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Health Assessment DRAFT Document for Carbon Tetrachloride

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HEALTH ASSESSMENT DOCUMENT FOR CARBON TETRACHLORIDE

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PREFACE

The Office of Health and Environmental Assessment, in consultation with an Agency work group, has prepared this health assesment to serve as a "source document" for Agency-wide use. Originally the health assessment was developed for use by the Office of Air Quality Planning and Standards, however, at the request of the Agency Work Group on Solvents, the assessment scope was expanded to address multimedia aspects. This assessment will help insure consistency in the Agency's consideration of the relevant scientific health data associated with carbon tetrachloride (CCl_4).

In the development of this assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summaries and conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and dose-response relationships are discussed evaluating the potential toxicity of CCl_4. Unit risk estimates for cancer are calculated to provide a media-specific measure of toxicity. This information can then be placed in perspective with observed environmental levels.

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1. INTRODUCTION

1.1. THE COMPOUND

Carbon tetrachloride (CCl_4), also known as perchloromethane or tetrachloromethane, is a haloalkane with a wide range of industrial and chemical applications. It is a volatile compound yet it is denser than water, thus rendering it quite stable under certain environmental conditions. Its oresence in the atmosphere and in water appears to be of anthropogenic origin due to its ubiquity. As would be expected from its partition coefficients, it is readily absorbed througn the lung and gastrointestinal tract and also through the skin.

Toxicological data for non-human mammals are extensive and indicate that CCl_4 causes liver and kidney damage primarily but also neurological damage and dermal effects. Case reports on humans document similar effects. The carcinogenicity of CCl_4 has been well-documented with both the International Agency for Research on Cancer and the National Cancer Institute identifying it as an animal carcinogen. It is a suspect human carcinogen.

2. SUMMARY AND CONCLUSIONS

2.1. SUMMARY

Carbon tetrachloride (CCl_4) is a relatively non-polar compound that is slightly soluble in water, soluble in alcohol and acetone, and miscible in benzene, chloroform and ether. Its density is 1.59 g/mℓ at 4°C which is greater than the density of water. Thus, under favorable conditions, large amounts spilled into water may settle and not volatilize. However, the high vapor pressure of CCl_4 (115.2 mm Hg at 25°C) favors volatilization from water to air.

CCl_4 is produced commercially from the chlorination of several chemicals such as methane, propane, ethane propylene and carbon disulfide. In 1980, 3.22×10^8 kg were synthesized in the U.S. This amount of CCl_4 is minimally supplemented indirectly during the production of vinyl chloride and perchloroethylene. The major use of CCl_4 is in the production of chlorofluorocarbons. A reduction in the use of CCl_4 has resulted in a 3.5% decrease in production over the years 1970 to 1980. A continued 1.0% decline in production is projected each year through 1985.

CCl_4 can be detected in the environment using media-specific analytical methods. Levels detected in the environment are generally <.01 mg/ℓ in water, <.01 mg/m^3 in air and <.01 mg/kg in food although higher levels have been detected on occasion in urban air and grain fumigated with CCl_4. Food products made from this grain also contain residues of CCl_4. Natural sources of CCl_4 are unknown so that most, if not all CCl_4 present in the environment can be accounted for by anthropogenic activities.

CCl_4 is extremely stable in the lower atmosphere and troposphere. However, once in the stratosphere, photodissociation is rapid. Its presence

in the stratosphere is of concern due to its possible contribution to the ozone-destroying chemical reactions and subsequent modification of UV-B radiation flux. Increased UV-B radiation studies in the laboratory have shown adverse affects to a variety of terrestrial plant species; for example, in terms of depressed photosynthetic activity, reduced growth rate, increased somatic mutation rates, and inhibition of seed germination. Some aquatic organisms have also been shown, in laboratory studies, to be adversely affected. Direct extension of these laboratory findings to the natural environment should not be made because of adaptability potential to small changes in UV-B flux. Indirect ecosystem effects associated with increases in UV-B radiation flux have been identified to include changes in genetic material and alterations in population composition. However, it is not known if these laboratory findings apply to the natural environment in lieu of observed adaptations. The implication to human populations is that >90% of skin cancer (other than melanoma) in the U.S. is attributed to sunlight in the UV-B region.

Under favorable conditions, which seldom occur, CCl_4 settles in water and, therefore, does not volatilize. In this case, it is extremely stable. In such capacity, the presence of CCl_4 in ambient water and drinking water may present a threat to aquatic ecosystems and human health.

Despite the fact that humans are potentially exposed to CCl_4 through various media, uptake from air appears to be the major source of exposure followed by liquids and food. For this reason, the fate and transport of CCl_4 has been most extensively studied in air. Information on CCl_4 soil contamination is limited, consequently, its contribution to human exposure is uncertain.

Ecological impacts have been monitored to a limited extent in freshwater and saltwater organisms. In two freshwater fish and one invertebrate species, 96-hour LC_{50}s were determined as low as 27.3 mg/ℓ in the bluegill (Lepomis macrochirus). Chronic toxicity data are not available. However, reported bioconcentration factors are <30 so that tissue residues are insignificant. The only data on saltwater species deals with toxicity following acute exposures. Effects have been identifed at levels of 50.0 mg/ℓ. It is noted that the toxic dose for both freshwater and saltwater species can be lower if a more sensitive species were tested.

In mammals, CCl_4 is readily absorbed from the lungs and gastrointestinal tract. Absorption also occurs through the skin but at a much slower rate. Following absorption, CCl_4 is distributed to all major organs. Metabolism of the compound occurs primarily in the liver where it is reduced to a trichloromethyl radical and thought to be further metabolized and/or released as a free radical. Excretion of CCl_4 is primarily through the lungs, but also occurs in the urine and feces.

Varying degrees of toxicity have been reported in humans and animals following acute, subchronic and chronic exposures via ingestion, inhalation or dermal administration. Such effects can occur systemically as well as locally. For example, cirrhosis of the liver has resulted from inhalation and dermal exposures, whereas lung lesions have resulted from oral ingestion. Animals surviving acute doses developed a range of effects such as damage to the liver, kidney, lung, and central nervous system as well as dermal effects; biochemical alterations were also noted. Animals receiving subchronic and chronic doses developed kidney and liver damage and, less frequently, damage to the central nervous system. It has been observed that exposure to a higher concentration over a shorter period of time produces a

greater effect upon the liver than exposure to a lower concentration over a longer period of time even though the product of time and concentration is equal in both cases.

In an attempt to verify the purity of CCl_4 used in the testing protocols of the studies in this document, it was observed that CCl_4 of imoure or technical grade was not reported.

Adverse effects seen in humans following CCl_4 exposure are similar to those seen in animals. Damage to the liver, kidney, lungs and central nervous system has been documented in various case reports. Biochemical alterations have been identified in case reports and one epidemiological study.

Exposure to CCl_4 did not produce skeletal or functional abnormalities but did result in signs of fetotoxicity. Rats exposed in utero to CCl_4 were noted to have fatty infiltration of the liver from days 1 to 4 after birth. CCl_4 is transferred to the neonate through mothers' milk. Other adverse reproductive effects include changes in testicular histology eventually resulting in functional infertility.

Carbon tetrachloride has consistently tested negative in the Salmonella assay. A positive mutagenic response was seen in an assay using Saccharomyces cerevisiae, however, there have been problems associated with this study.

Numerous studies report on the carcinogenic effects of CCl_4 on experimental animals. Both the National Cancer Institute which uses CCl_4 as a positive control in some of its bioassays, and the International Agency for Research on Cancer have concluded that it is a carcinogenic substance to experimental animals. The studies on experimental animals indicate that CCl_4 is carcinogenic to three species: hamsters, mice and rats in order of decreasing sensitivity.

Case reports of human carcinomas developing years after a history of high CCl_4 exposures offer little conclusive evidence of human carcinogenicity. However, they are consistent with the carcinogenic potential of CCl_4 as suggested by animal studies.

In assessing toxicity, carcinogenicity or any other harmful effect, compounds that react synergistically or antagonistically with CCl_4 must be considered. Identified synergistic substances include ethanol, Kepone, PCB and PBB. Antagonistic effects have been demonstrated with such compounds as chloramphenicol and catechol.

2.2. CONCLUSIONS

Carbon tetrachloride causes damage to the liver, lungs, kidneys and central nervous system in humans. These effects are primarily the result of high oral or inhalation exposures. Less severe effects such as biochemical alterations, nausea and headache result from lower exposures or are secondary to the major health hazards attributed to higher exposures. Similar responses have been demonstrated in animals. These animal studies provide useful dose/response data, are well-defined and can identify a causal relationship between the CCl_4 insult and the toxic response. Furthermore, the toxicity from CCl_4 is not only local but also systemic.

Absorption of CCl_4 varies with species. Based upon both human and animal data, absorption coefficients of 40% when route of exposure is via inhalation and 100% when route of exposure is via ingestion are recommended.

The potential exists for embryotoxicity, especially in males. Toxic effects due to CCl_4 have been demonstrated in mammalian fetuses and neonates exposed directly or indirectly via the placenta or mothers' milk, respectively. Teratogenic effects have not been noted following CCl_4 exposure, however, degenerative changes in the testes and subsequent infertility of the offspring have occurred.

Definitive conclusions concerning mutagenicity tests cannot be reached. CCl_4 did exhibit a positive mutagenic response in an assay using Saccharomyces cerevisiae, however, due to problems associated with the study and the lack of corroborative studies, the evidence is not adequate to conclude whether or not CCl_4 is genotoxic.

Interactions with other chemicals must be considered in assessing the potential health hazards of exposure to CCl_4. Chemicals have been identified that potentiate the effects of CCl_4 as well as those that inhibit the effects of CCl_4.

Carcinogenicity of CCl_4 has been observed in three animal species. The primary lesions are hepatic neoplasms. Cirrhosis, necrosis and cholangiofibrosis have also been found and have been suggested as initial lesions prior to tumorigenesis in liver. Human data on carcinogenicity are restricted to case reports and one preliminary epidemiological study. The animal data provide evidence to indicate that carbon tetrachloride is a potential human carcinogen.

Mechanism of action is a fundamental issue in the assessment of the potential carcinogenicity of carbon tetrachloride to humans at low doses. The possibility exists that carbon tetrachloride acts through a nongenotoxic mechanism to produce a carcinogenic effect; evidence in favor of this possibility includes signs of liver toxicity observed along with liver tumor formation in positive animal carcinogenicity studies and the primarily negative results from currently available mutagenicity studies. However, Eschenbrenner and Miller (1946) reported liver tumor development without concomitant necrosis in treated mice in their carcinogenicity study, and in their carcinogenicity study with five strains of rat, Reuber and Glover (1970) found greater liver tumor incidence in strains showing lower severity of

cirrhosis. Furthermore, as indicated in the mutagenicity section herein, currently available mutagenicity studies do not provide a conclusive judgment on the mutagenic potential of carbon tetrachloride. Further investigation, particularly in regard to genotoxic potential, is indicated to elucidate the carcinogenic mechanism of action for carbon tetrachloride.

2.2.1. Major Research Needs.

- Experiments in a number of species designed to derive an absorption coefficient or an absorption range are needed.
- Definitive data on CCl_4-induced carcinogenicity and toxicity in humans including the mechanism of action are needed. While results on short-term exposures are available, they quite often do not contain adequate dose information. Epidemiology studies on occupational groups are warranted as demonstrated in the preliminary study on drycleaners.
- Long-term studies on animals exposed in utero are needed to assess lifetime effects of such exposures.
- Chronic studies on animals exposed to CCl_4 via drinking water are needed to establish a dose-response relationship.
- The toxicity data on the rat and guinea pig are satisfactory. Additional data on mice are needed since, at present, dose/response information is inadequate. Studies on other species would also be useful.
- Toxic effects of chronic exposure on freshwater and saltwater organisms need to be documented.
- Assessment of the overall ecological impact is sparse. Information on soil and air, particularly the stratosphere (levels, fate and transport processes, relative source contribution) are needed, as well as bioaccumulation/bioconcentration in shellfish.
- Additional research to better define the genotoxic potential and epigenetic potential of CCl_4.

3. CHEMICAL AND PHYSICAL PROPERTIES/ANALYTICAL METHODOLOGY

3.1. CHEMICAL AND PHYSICAL PROPERTIES

Carbon tetrachloride (CCl_4) is a clear, colorless, nonflammable liquid with a characteristic odor (Windholz, 1976). It is a relatively nonpolar compound that is slightly soluble in water (0.8 g/ℓ at 25°C) (Johns, 1976), soluble in alcohol and acetone, and miscible in benzene, chloroform and ether (Weast, 1978).

Carbon tetrachloride may be quite stable under certain environmental conditions. An estimated 70,000 years are required for half of a given quantity of CCl_4 to decompose in water (Johns, 1976). This decomposition rate is considerably accelerated in the presence of metals such as iron (Pearson and McConnell, 1975). However, hydrolytic decomposition as a means of removal from water appears to be insignificant as compared to evaporation, since the properties (Table 3-1) of carbon tetrachloride favor volatilization of the compound from water to air. Carbon tetrachloride has a high vapor pressure (115.2 mm Hg at 25°C) (Johns, 1976). The air/water partition coefficient of carbon tetrachloride at 20°C is 1.1 by volume and about 1000 by weight (Johns, 1976). The rapid vaporization predicted from these properties has been confirmed by Dilling et al. (1975), who reported a half-life of carbon tetrachloride evaporation of 29 minutes from a dilute aqueous solution at 25°C.

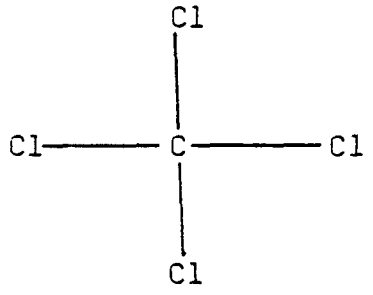
The density of carbon tetrachloride in water is 1.59 g/mℓ at 4°C (Weast, 1978). Because its density is greater than the density of water, some carbon tetrachloride from large spills in water might tend to settle before it is totally dispersed, emulsified or volatilized.

Volatilization is the major transport process for removal of CCl_4 from aquatic systems. Once in the troposphere, CCl_4 remains stable; it

TABLE 3-1

Physical and Chemical Properties
of Carbon Tetrachloride

A. Structure



B. Synonyms

Tetrachloromethane
Methane tetrachloride
Perchloromethane
Benzinoform
Necatorina

C. Registry Numbers

CAS No. 56-23-5
TSL No. FG 4900000

D. Description

Carbon tetrachloride is a clear, colorless, nonflammable liquid with a charcteristic odor (Windholz, 1976). It is slightly soluble in water, soluble in alcohol and acetone, and miscible in benzene, ether and chloroform (Weast, 1978).

E. Physical Properties		Reference
1. Molecular weight	153.82	Weast, 1978
2. Melting point	-22.9°C	Weast, 1978
3. Boiling point at 760 torr	76.54°C	Weast, 1978
4. Density in water at 4°C	1.594 g/mℓ	Weast, 1978
at 25°C	1.589 g/mℓ	Windholz, 1976
5. Vapor pressure at 25°C	115.2 mm Hg	Weast, 1978
6. Solubility in water 20°C	785 mg/ℓ	Pearson and McConnell, 1975
7. Log octanol/water partition coefficient	2.64	Neely et al., 1974
8. Conversion factors at 20°C 1 atm.	0.156 ppm for 1 mg/m^3	Verschuren, 1977
	6.402 mg/m^3 for 1 ppm	

exhibits an extremely slow rate of reaction with hydroxyl radicals present in the troposphere. The atmospheric lifetime of CCl_4 is 30 to 100 years. CCl_4 eventually diffuses into the stratosphere or is carried back to the earth during the precipitation process. Once in the stratosphere, CCl_4 is degraded on exposure to shorter wavelength, higher energy ultraviolet light to eventually form phosgene as the principal initial product (44 FR 68624-68707).

3.2. ANALYTICAL METHODOLOGY

3.2.1. Carbon Tetrachloride in Water.

3.2.1.1. SAMPLING -- Grab samples must be collected in glass containers having a total volume in excess of 40 ml. The sample bottles should be filled in such a manner that no air bubbles pass through the sample as the bottle is being filled. The bottle should then be sealed so that no bubbles are entrapped in it. The hermetic seal should be maintained until the time of analysis.

The samples must be iced or refrigerated from the time of collection until extraction. If the sample is known to contain free or combined chlorine, sodium thiosulfate preservative (10 mg/40 mℓ will suffice for up to 5 mg/ℓ Cl_2) should be added to the empty sample bottles just prior to shipping to the sampling site. In collection of the sample, the bottle should be filled just to overflowing. After sealing the bottle, the sample should be shaken vigorously for 1 minute. All samples must be analyzed within 14 days of collection (44 FR 68624-68707).

3.2.1.2. ANALYSIS

3.2.1.2.1. Ambient Water -- Carbon tetrachloride (and 47 other halogenated organics) in water can be analyzed by a purge and trap method (Method 502.1) described by the U.S. EPA Environmental Monitoring and Support Laboratory (U.S. EPA, 1980b). This method can be used to measure

purgeable organics at low concentrations. Purgeable organic compounds are trapped on a Tenax GC-containing trap at 22°C using a purge gas rate of 40 mℓ/min for 11 minutes. The trapped material is then heated rapidly to 180°C and backflushed with helium at a flow rate of 20 to 60 mℓ/min for 4 minutes into the gas chromatographic analytical column. The programmable gas chromatograph used is capable of operating at 40°±1°C. The primary analytical column is stainless steel packed with 1% SP-1000 on Carbopack B (60/80) mesh (8 ft x 0.1 in. I.D.) and is run at a flow rate of 40 mℓ/min. The temperature program sequence begins at 45°C for 3 minutes, increases 8°C/min to 220°C, and is then held constant for 15 minutes or until all compounds have eluted. A halogen-specific detector with a sensitivity to 0.10 µg/ℓ and a relative standard deviation of 10% must be used. The optional use of gas chromatography/mass spectometry (GC/MS) techniques of comparable accuracy and precision is acceptable.

Carbon tetrachloride can also be detected in water using the headspace gas chromatography method in conjunction with electron capture detection (GC/ECD) described by Dietz and Singley (1979). A 10 ft. by 4 mm (i.d.) glass column was used containing 20% SP-2100/0.1% Carbowax 1500 on 100/120 mesh Supelcoport. With this method, CCl_4 and other chlorinated hydrocarbons can be detected from 0.1 µg/ℓ to the low mg/ℓ range at ambient temperatures. The headspace method relies on the fact that when a water sample containing organic compounds is sealed in a vial, the organics will equilibrate between the headspace in the vial and the water (Dietz and Singley, 1979). Distribution of the compounds between the two media depends on a number of physical and chemical parameters. With these parameters known, specific measurements can be made on the volatile compounds of interest. Although it is not clearly stated by the authors, accuracy $\geq$98% can be obtained, and assuming a constant and additive term, precision = 0.1.

3.2.1.2.2. **Municipal and Industrial Discharges** -- The proposed method is designed to be used to meet the National Pollutant Discharge Elimination System (NPDES). In this regard, it presupposes a high expectation of finding the specific compounds of interest. It can be utilized to screen samples; however, the user must develop an independent protocol for the verification of identity.

The method of analysis is gas chromatography (GC) and high performance liquid chromatography (HPLC) for purgeable halocarbons. HPLC has been developed considerably in the past few years and can be used to achieve separations and measurements that cannot be performed with state-of-the-art GC. In short, the method has been developed for the measurement of solvents and other volatile materials using variations of the Bellar purge and trap technique. Semispecific detectors are used to minimize background interferences. A detailed description of the method is provided in the Federal Register, December 3, 1979 (44 FR 68624-68707)

The sensitivity of this method is usually dependent upon the level of interferences rather than instrumental limitations. The limit of detection for carbon tetrachloride is 0.007 µg/ℓ and represents the sensitivity that can be achieved in wastewaters under optimum operating conditions.

3.2.2. **Carbon Tetrachloride in Air.**

3.2.2.1. **SAMPLING** -- Ambient air sampling of CCl_4 can involve its adsorption onto a suitable medium such as Tenax GC. Recovery can then be accomplished by thermal desorption and purging with helium into a liquid nitrogen cooled nickel capillary trap (Pellizzari and Bunch, 1979). This method is the most common and is specific to analysis by GC. Sampling techniques will vary with the analytical methodology.

3.2.2.2. **ANALYSIS** -- Many of the techniques used in analyzing CCl_4 in water apply to air. There are four practical methods to measure air concentrations of CCl_4 [National Academy of Sciences (NAS), 1978]. They are (1) GC/ECD; (2) GC/MS; (3) long path infrared absorption spectroscopy, usually with preconcentration of whole air and then separation of the compounds by gas chromatography (GC/IR); and (4) infrared solar spectroscopy (NAS, 1978).

GC/ECD is by far the most widely used method (Lovelock et al., 1973; Lillian et al., 1975; Penkett et al., 1979). The instrumentation needed for this method is readily obtained and relatively inexpensive, costing between $5000 and $10,000 (NAS, 1978). It is sturdy and easy to operate and quite sensitive for CCl_4, which makes it ideal for use on aircraft (Sandalls and Hatton, 1977) and ships.

The use of GC/MS has been more recent (Grimsrud and Rasmussen, 1975; Barkley et al., 1980; Dmitriev et al., 1980). Although equally as sensitive as the GC/ECD method, GC/MS has the ability to positively identify compounds by their characteristic mass spectra, whereas GC/ECD must rely upon the somewhat imprecise method of retention times to identify compounds (NAS, 1978). Unfortunately, GC/MS instrumentation is quite expensive costing $70,000 or more (NAS, 1978). The method employed by Dmitriev et al. (1980) undertakes the chromatographic separation at room temperature for the first 5 minutes, then at a rising temperature to 150°C at 5°C/minute intervals for a total chromatography time of approximately 30 minutes, using adsorbants such as Tenax or Polysorbamide. The chromatographic fractions are then analyzed by MS. The authors report a sensitivity level of 1 $\mu g/m^3$ with this method (Dmitriev et al., 1980).

GC/IR has the advantage of real-time continuous measurements; however, the disadvantage of poor sensitivity renders this method less desirable

(NAS, 1978). Furthermore, the method is expensive, costing $20,000-$100,000 and cannot be used in the field (NAS, 1978). This method is used and reported by Hanst et al. (1975) with a detailed description of sample collection techniques.

Finally, infrared solar spectroscopy has been described by Rasmussen, 1976 and Murcray et al., 1975 (NAS, 1978). This method uses the solar spectrum passing through the atmosphere at large zenith angles to obtain the necessary path length to give sufficient absorption to detect ambient halocarbon levels. Although not in real-time, this method provides continuous data on a remote region of the atmosphere (NAS, 1978), i.e., the stratosphere. However, it is limited to that region alone.

3.2.3. Carbon Tetrachloride in Soil. Little research has been done to detect CCl_4 in soil; however, with concern increasing regarding leachates from landfills and waste disposal sites, the analysis of soil samples for organic compounds has become more important as an indicator of possible ground water contamination. A recent article by DeLeon et al. (1980) describes a method of analysis for volatile and semivolatile organochloride compounds.

3.2.3.1. **SAMPLING** -- In the method described by DeLeon et al. (1980), samples were taken from 30 ft. vertical borings using the splitspoon method. The samples were then placed in jars and sealed with Teflon-lined screw caps. During shipment, they were maintained at 6 to 10°C. Upon their arrival at the analysis site, they were maintained at -20°C until prepared for analysis.

3.2.3.2. **ANALYSIS** -- The analytical method described (DeLeon et al., 1980) employs a simple extraction procedure using hexane followed by analysis of the extract using temperature programmed GC on high-resolution glass

capillary columns with ECD. The electron capture gas chromatographic results are confirmed by mass spectometry much like the method used for water sample analysis. Reported sensitivity is at least 10 µg/g. The authors demonstrate this technique to be effective in determining the perimeter and limits of an old chemical waste disposal site and also to be an effective method to assess the extent of leaching of chlorocarbons from the waste disposal site into surrounding soils (DeLeon et al., 1980).

3.3. SUMMARY

Carbon tetrachloride (CCl_4) is a clear, colorless, nonflammable liquid with a characteristic odor and is slightly soluble in water. Its high vapor pressure favors rapid volatilization from water to air. This characteristic is utilized in most commonly accepted methods of analysis for CCl_4. Gas chromatography (GC), either alone or coupled with mass spectroscopy (MS), is the most widely used detection method. The compound is usually separated from other constitutents in the sample by direct volitilization, extraction or heating, and then analyzed using GC, GC/MS or some other analytical technique.

4. PRODUCTION, USE AND ENVIRONMENTAL EXPOSURE LEVELS

4.1. PRODUCTION

Carbon tetrachloride is produced industrially by the chlorination of methane, propane, ethane, propylene or carbon disulfide (Rams et al., 1979). In 1980, 322 million kg were synthesized [U.S. International Trade Commission (USITC), 1981]. Carbon tetrachloride is also produced indirectly during the production of compounds such as vinyl chloride and perchloroethylene (Rams et al., 1979).

The demand for carbon tetrachloride in the U.S. is projected at 716 million pounds in 1980, 708 million pounds in 1981 and 680 million pounds in 1985 (Chemical Marketing Reporter, 1981). This represents a 1.0% decline per year through 1985, while the historical trend over the last 10 years (1970 to 1980) is a decline of 3.5% per year. Carbon tetrachloride production in the U.S. has declined at a rate of 7.9% per year since reacning its peak level of 1974.

Production levels have decreased as a result of the rapid decline in its major end product area, fluorocarbon aerosols. In 1975 fluorocarbon aerosols had a 50% share of the domestic aerosol market; however, this declined to 20% in 1977 due to the ozone depletion controversy (Chemical Marketing Reporter, 1981). Fluorocarbon aerosols are expected to show some strength in refrigerant and foam-blowing applications. Growth in these areas is expected to stabilize carbon tetrachloride production in the 1980s.

4.2. USE

Currently the major use of carbon tetrachloride is in the production of chlorofluorocarbons, which are used as refrigerants, foam-olowing agents and solvents. These uses accounted for 95% of the total U.S. consumption in 1980. Carbon tetrachloride is also used in fumigants and has a variety of

minor uses (5% of total 1980 consumption), including those as a solvent in metal cleaning and in manufacture of paints and plastics (Rams et al., 1979). It is being replaced in grain fumigation by other registered pesticide products (U.S. EPA, 1980a), and its registration for use in fumigants is presently under review by U.S. EPA (45 FR 68534-68584). Approximately 12% of the total 1980 production was exported.

4.3. ENVIRONMENTAL EXPOSURE LEVELS

Carbon tetrachloride present in the environment appears to be of anthropogenic origin (Singh et al., 1976). Its presence in surface waters occurs primarily as a result of industrial and agricultural activities although some may reach surface water through rainfall. Groundwater contamination may be the result of leaching from solid waste sites. This is also a source of soil contamination. Air is the media wherein the greatest concentrations of CCl_4 can be found in the environment. The major source of CCl_4 in air is industrial emission. Once in the air, CCl_4 can be washed into surface waters and soil through rainfall.

Once in the environment, carbon tetrachloride is relatively stable. Its half-life for hydrolytic breakdown in water at pH 1.0 to 7.0 is estimated to be 70,000 years, but hydrolysis appears to be accelerated in the presence of metals such as iron and zinc (Johns, 1976). The high stability in water has little practical significance, however, since carbon tetrachloride vaporizes readily to air. The atmospheric lifetime of carbon tetrachloride appears to be on the order of 30 to 100 years (Singh et al., 1976).

The presence of carbon tetrachloride in the environment is of concern for two reasons. As indicated by both animal and human studies, CCl_4 may pose a health problem through direct exposure in the air, water, food and/or

soil. However, CCl_4 may also contribute to ozone-destroying photochemical reactions in the stratosphere. This might cause increases in the incidence of human skin cancers and animal cancers, affect terrestrial and aquatic ecosystems, and bring about climatic changes (NAS, 1978, 1982).

Although levels of carbon tetrachloride in the environment are generally in the low ppb range or below (NAS, 1978), carbon tetrachloride may pose a long-term danger because of its possible carcinogenic potential (see Chapter 11). In urban and industrial areas where higher concentrations of carbon tetrachloride in air occur, other toxic effects may result (e.g., liver and renal damage).

No natural sources of carbon tetrachloride have been reported. The presence of a natural source was suggested because of the large quantity of carbon tetrachloride in the atmosphere and the homogeneity of ambient concentrations in both hemispheres (Lovelock et al., 1973). However, this suggestion has since been challenged, since estimates of cumulative worldwide production and emissions of carbon tetrachloride appear to account for the carbon tetrachloride found in the environment (Singh et al., 1976).

4.3.1. Possible Sources and Levels of Carbon Tetrachloride in Water. Carbon tetrachloride has been monitored extensively in drinking water and, to a lesser extent, in natural waters. The chemical's concentration in drinking water has been reported as <.007 mg/ℓ (Symons et al., 1975). Samples of ocean, lake and ground water have generally yielded carbon tetrachloride concentrations in the ppt range. There are some indications that industrial activity may lead to increased carbon tetrachloride concentrations in surface and ground water. These monitoring studies are discussed below.

In the National Organics Reconnaissance Survey (NORS), U.S. EPA found carbon tetrachloride levels of <.003 mg/ℓ in drinking water in 80 cities (Symons et al., 1975). The more recent National Organics Monitoring Survey (NOMS) of 113 public drinking water systems detected carbon tetrachloride in the range of .0024 to .0064 mg/ℓ in 10% of the samples surveyed (U.S. EPA, 1980a). Carbon tetrachloride concentrations in these samples were very low compared to those of chloroform and other organics.

Carbon tetrachloride has been detected in drinking water in Tuscaloosa, Alabama (Bertsch et al., 1975); the District of Columbia (Scheiman et al., 1974); Durham, North Carolina (McKinney et al., 1976); and New Orleans, Louisiana (Dowty et al., 1975). In the District of Columbia, carbon tetrachloride in drinking water was measured at .005 mg/ℓ (Scheiman et al., 1974). In New Orleans, higher concentrations of carbon tetrachloride were found in blood plasma than in drinking water, suggesting to the authors the presence of a bioaccumulation mechanism or sources of the compound other than drinking water (Dowty et al., 1975). The former, however, has not been demonstrated. Carbon tetrachloride was also found in drinking water in Germany in the ng/ℓ range (Sonneborn and Bohn, 1977).

Under unusual conditions, carbon tetrachloride may be found at high levels in raw and drinking water. After a chemical manufacturer accidentally spilled an estimated 70 tons of carbon tetrachloride into the Kanawha River, the U.S. EPA determined that raw Ohio River water contained carbon tetrachloride levels up to .340 mg/ℓ; drinking water levels were found to be as high as .1 mg/ℓ (Landen, 1979). As indicated by this incident, levels of CCl_4 detected in ambient water should not be construed to be the same levels of CCl_4 in public drinking water supplies. The treatment process does remove some of the contaminant.

Carbon tetrachloride has been found in the ppb range or lower in samples of rain, surface water, potable water and seawater (McConnell et al., 1975). Trace levels of carbon tetrachloride have been reported in snow (Su and Goldberg, 1976). Carbon tetrachloride was also detected in the Atlantic Ocean at mean levels of 60 ppt (±17 ppt). Ocean levels of carbon tetrachloride were only slightly higher in the Northern Hemisphere than in the Southern Hemisphere (Lovelock et al., 1973).

Lake Zurich and the ground water in an industrial section of Zurich, Switzerland, were also monitored for carbon tetrachloride. At various depths of Lake Zurich, concentrations of this compound of approximately 25 ppt were measured, with no significant variation. Ground water levels of carbon tetrachloride in the industrial sector were much larger. The compound was detected in 4 of 18 samples at levels ranging between 190 to 3600 ppt (Giger et al., 1978).

Carbon tetrachloride can be emitted to the environment through the production and use of the chemical, and through the production and use of chlorofluorocarbons and other chlorinated compounds that contain carbon tetrachloride impurities. Although small amounts of carbon tetrachloride may be directly released to water systems through these processes, most of the carbon tetrachloride emitted to the environment has been estimated to be released to air or land (Rams et al., 1979). Direct releases to water may not account for the carbon tetrachloride eventually detected in water. The chemical may find its way from air or land to surface and ground water systems through rainfall, runoff from agricultural sites, dumping sites or industrial sites, and landfill leaching.

High levels of carbon tetrachloride in the ground waters of Zurich, Switzerland have been attributed to industrial processes in the Zurich area (Giger et al., 1978). Levels of carbon tetrachloride would be expected to

be highest in industrialized areas because of both industrial and consumer use of carbon tetrachloride and its products in these areas.

Carbon tetrachloride does not appear to be produced in water through chlorination reactions, unlike other chlorinated organics such as chloroform (44 FR 68624-68707). Recent high levels of carbon tetrachloride detected in Philadelphia drinking water following chlorination ($\leq$0.046 mg/ℓ) were found to be the result of the use of carbon tetrachloride-contaminated chlorine. This incident resulted in a meeting between U.S. EPA and the Chlorine Institute during which an interim maximum level for carbon tetrachloride in chlorine for drinking water use was set at 0.1 mg/ℓ. The use of chlorine of this purity should result in carbon tetrachloride levels of <0.001 mg/ℓ in drinking water (U.S. EPA, 1977).

4.3.2. Possible Sources and Levels of Carbon Tetrachloride in Air. Reported concentrations of carbon tetrachloride in continental and marine air masses in 1978 were very similar (0.00070 to 0.00084 mg/m^3) (Table 4-1). As reported by Lovelock et al. (1973), the levels of carbon tetrachloride in the Southern Hemisphere are only slightly lower than those in the Northern Hemisphere. The homogeneity of ambient carbon tetrachloride concentrations throughout the atmosphere has been attributed to its slow and continual release to the atmosphere for many years (45 FR 68534-68584).

In general, carbon tetrachloride levels over urban areas are only slightly higher than background levels (Table 4-1). However, some higher concentrations have been reported. An average annual concentration of 0.0088 mg/m^3 was reported over Tokyo in 1974-75 (Ohta et al., 1976). This level was the highest measured over an extended period of time. Other high ambient concentrations measured reflected one sample or several covering a short time period (NAS, 1978). The maximum concentration ever detected was

TABLE 4-1

Summary of Atmospheric Concentrations of Carbon Tetrachloride*

Type of Measurement	Carbon Tetrachloride mg/m^3
Continental background	0.00076 ± 0.00008
	0.00084 ± 0.00006
	0.00073 ± 0.00005
	0.00075 ± 0.00009
Marine background	0.00081 ± 0.00003
	0.00081 ± 0.00010
	0.00070 ± 0.00007
Urban range	0.00084 ± 0.00012
	0.00075 ± 0.113
	0.0088
	0.00075 ± 0.0094

*Source: NAS, 1978

0.113 mg/m^3 in Bayonne, New Jersey (Lillian et al., 1975). A carbon tetrachloride concentration of >0.0094 mg/m^3 was also detected in the air of Grenoble, France (Su and Goldberg, 1976). In 1980, Singh et al. measured CCl_4 concentrations in seven U.S. cities over 2-week periods (Table 4-2). The highest level detected using GC/ECD was 0.0188 mg/m^3 over Houston, Texas.

Carbon tetrachloride in the atmosphere appears to be of anthropogenic origin, because estimates of releases of carbon tetrachloride from industrial processes and uses appear to account for the amount present in the atmosphere (Singh et al., 1976; Penkett et al., 1979). In 1978, approximately 4.5 million pounds of CCl_4 were emitted from production facilities. The total nationwide emissions of CCl_4 in 1978 from all sources were estimated at 65 million pounds. The primary source of these emissions is solvent application.

4.3.3. Possible Sources and Levels of Carbon Tetrachloride in Food. In British studies, carbon tetrachloride was found in various foods as follows (McConnell et al., 1975; Pearson and McConnell, 1975):

	Concentration (mg/kg)
Dairy products	.0002 - .014
Meat	.007 - .009
Oils and fats	.0007 - .018
Beverages	.0002 - .006
Fruits and vegetables	.003 - .008
Black grapes (imported)	.0197
Fresh bread	.005
Fish and seafood	.0001 - .006

TABLE 4-2

Atmospheric Concentrations of Carbon Tetrachloride
Over Seven U.S. Cities*

City/State	Sampling Dates	CCl_4 (mg/m^3)		
		Mean	Max	Min
Los Angeles, CA	Apr. 9 - Apr. 21, 1979	.0014	.0064	.0006
Phoenix, AZ	Apr. 23 - May 6, 1979	.0018	.0055	.0008
Oakland, CA	June 28 - July 10, 1979	.0011	.0063	.0006
Houston, TX	May 14 - May 25, 1980	.0026	.0188	.0008
St. Louis, MO	May 29 - June 6, 1980	.0008	.0009	.0007
Denver, CO	June 15 - June 28, 1980	.0011	.0018	.0007
Riverside, CA	July 1 - July 13, 1980	.0011	.0017	.0010

*Source: Singh et al., 1980

The best studied mechanism of carbon tetrachloride contamination in food is its use as a fumigant for grain. Much higher carbon tetrachloride concentrations than those reported above have been found in fumigated grains, as high as 115 mg/kg in wheat and 21 mg/kg in flour (Lynn and Vorches, 1957). Carbon tetrachloride levels decline dramatically in bread baked from fumigated wheat with residual levels generally reported as $\leq$10 mg/kg (U.S. EPA, 1980a; Jagielski et al., 1978), although contamination as high as 0.13 mg/kg was reported in one study (Berek, 1974). The studies of carbon tetrachloride levels in fumigated grains and grain products are discussed below.

Wheat, corn and milo fumigated and stored for 1 to 6 months were shown to contain carbon tetrachloride at 2.9 to 20.4 mg/kg. The levels of carbon tetrachloride detected appeared to be dependent upon length of storage and concentration of fumigants (McMahon, 1971). In a monitoring program for grain imported into the Netherlands, carbon tetrachloride was detected in half of the cereals sampled. Of these samples, 3% contained >5 mg/kg carbon tetrachloride (Wit, 1972). The U.S. EPA Pesticide Laboratory detected .005 to 2.61 mg/kg carbon tetrachloride in flour from 11 U.S. cities, with an average level of .051 mg/kg (45 FR 68534-68584). Carbon tetrachloride levels of .0002 to .0003 mg/kg were detected in flour in another study. However, bread and biscuits made from this flour contained undetectable carbon tetrachloride (<5 mg/kg) (Bondi and Alumot, 1972).

In several experiments, researchers have simulated commercial fumigation conditions to determine residual levels of carbon tetrachloride in foods. Fumigated wheat, aerated for several weeks, was found to contain 20 to 62 mg/kg carbon tetrachloride. Flour made from this wheat was found to contain 2 to 10 mg/kg carbon tetrachloride, whereas white bread made from the flour contained $\leq$0.007 mg/kg (Wit et al., 1972). In another study, carbon

tetrachloride at 200 to 400 mg/kg was detected in wheat and corn after application of a fumigant (Scudamore and Heuser, 1973). Residual carbon tetrachloride decreased to 1 to 10 mg/kg 6 months after fumigation. By 12 months after fumigation, the wheat and corn contained a maximum of 4.7 mg/kg of CCl_4. Wheat and barley were analyzed for carbon tetrachloride in a study by Bielorai and Alumot (1966). The initial carbon tetrachloride concentrations of 1.53 mg/kg in wheat and 2.2 mg/kg in barley decreased to 0.7 and 0.6 mg/kg, respectively, by day 17.

Carbon tetrachloride was detected in levels of 76 to 115 mg/kg in wheat, 10 to 21 mg/kg in flour, 28 to 39 mg/kg in oats and 43 to 88 mg/kg in bran that had been fumigated with recommended fumigant dosages (Lynn and Vorches, 1957). In another study, carbon tetrachloride levels were determined in fumigated wheat and wheat fractions and in bread prepared from the wheat (Berck, 1974). Levels in wheat ranged between 3.2 mg/kg (7 weeks of aeration) and 72.6 mg/kg (1 week of aeration). Flour, bran and middlings contained 0.2 to 0.93, 0.43 to 3.53 and 0.2 to 1.65 mg/kg, respectively. Bread contained $\leq$0.13 mg/kg carbon tetrachloride. In a similar study, flour treated at normal fumigant levels was found to contain carbon tetrachloride at levels of 0.6 to 1.6 mg/kg; levels in bran were 2.9 to 5.3 mg/kg (Jagielski et al., 1978). Bread baked from the flour contained <.01 mg/kg carbon tetrachloride. Results of these studies indicated that the amount of carbon tetrachloride remaining as a residue is dependent upon the fumigant dosage, storage conditions, length of aeration and extent of processing.

In addition to its use as a grain fumigant, carbon tetrachloride may find its way into other foods through the use of herbicides, insecticides and fungicides containing carbon tetrachloride as a contaminant (0.18 to 0.4%) in the pesticide formulation. Food may also become contaminated by carbon tetrachloride in the air (45 FR 68534-68584).

4.3.4. **Possible Sources and Levels of Carbon Tetrachloride in Soil.** Carbon tetrachloride can occur in soil due to spills, runoff and leaching. As in ground water contamination, CCl_4 may find its way into the soil by runoff from agricultural, dumping and industrial sites and through landfill leaching.

Waste water treatment of night soil in Japan also resulted in CCl_4 formation. Methanol (MeOH)-water-solution substance was fractionated along with humic, fulvic and hymatomelanic acids from the effluent of the night soil treatment plant by the use of Amberlite XAD-2. Each of these four fractions was chlorinated at pH 7.0. Although all fractions primarily produced $CHCl_3$, CCl_4 was formed by chlorination of the MeOH-water-solution substance (Ishikawa et al., 1978).

4.4. RELATIVE SOURCE CONTRIBUTIONS

The widespread distribution of carbon tetrachloride in the environment can lead to exposure to the chemical through water, food and air. Quantities of carbon tetrachloride potentially taken into the body as a result of exposure to carbon tetrachloride in air, water and food were estimated by the National Academy of Sciences (1978). Occupational exposure to carbon tetrachloride was not considered in these estimates. The NAS computations were based upon data for fluid consumption and respiratory volume for reference individuals and worldwide per capita food consumption as compiled by the International Commission for Radiological Protection (ICRP, 1975). These data are shown in Tables 4-3 through 4-7.

Data on measured fluid intakes for adults and individuals are presented in Table 4-3, and calculated intakes of milk, water and other fluids for a typical (reference) man, woman, 10-year-old child and 1-year-old infant are given in Table 4-4. Respiratory volumes for these reference individuals are

TABLE 4-3

Measured Fluid Intakes[a]

	Total Fluids[b]		Milk		Tap Water		Water-Based Drinks[c]	
	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)
Adults (normal conditions)	1000-2400	365-876	120-450	44-164	45-730	16.4-266	320-1450	117-529
Adults (high environmental temperature to 32°C)	2840-3410 3256±900	1037-1245 1188±329	-	-	-	-	-	-
Adults (moderately active)	3700	1351	-	-	-	-	-	-
Children (5-14 yrs)	1000-1670	365-610	330-650	120-237			540-790 mℓ/day[d] 197-288 ℓ/yr[d]	

[a]Source: Adapted from ICRP, 1975

[b]Numbers in these columns are independent measurements, not totals from the following six columns. Totals of milk, tap water, and water-based drinks, therefore, do not correspond to total fluid values.

[c]Includes tea, coffee, soft drinks, beer, cider, wine, etc.

[d]Combined tap water and water-based drinks.

TABLE 4-4

Fluid Intake for Reference Individuals*

	Adult Man		Adult Woman		Child, 10 yr	
	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)	(mℓ/day)	(ℓ/yr)
Milk	300	109.5	200	73.0	450	164.3
Tap water	150	54.8	100	36.5	200	73.0
Other	1500	547.5	1100	401.5	750	273.8
Total fluid	1950	711.8	1400	511.0	1400	511.0

*Source: Adapted from ICRP, 1975

TABLE 4-5

Respiratory Volumes for Reference Individual*
(in liters of air breathed)

	Adult Man	Adult Woman	Child, 10 yr	Infant, 1 yr
8 hr working, light activity	9600	9100	6240	2500 (10-hr)
8 hr nonoccupational	9600	9100	6240	
8 hr resting	3600	2900	2300	1300 (14-hr)
Total per day	2.3×10^4	2.1×10^4	1.5×10^4	0.38×10^4
Total per year	8.4×10^6	7.7×10^6	5.5×10^6	1.4×10^6

*Source: Adapted from ICRP, 1975

TABLE 4-6

Per Capita Estimates of World Food Consumption by Region[a] (g/day)

Food Group	Far East	Near East	Africa	Latin America	Europe	North America	Oceania
Cereals[b]	404	446	330	281	375	185	243
Starchy roots[c]	156	44	473	247	377	136	144
Sugar[d]	22	37	29	85	79	113	135
Pulses & nuts[e]	56	47	37	46	15	19	11
Vegetables & fruits[f]	128	398	215	313	316	516	386
Meat[g]	24	35	40	102	111	248	312
Eggs[h]	3	5	4	11	23	55	31
Fish[i]	27	12	16	18	38	26	22
Milk[j]	51	214	96	240	494	850	574
Fats & oils[k]	9	20	19	24	44	56	45

[a]Source: Adapted from ICRP, 1975
[b]Flour and milled rice content.
[c]Includes sweet potatoes, cassava and other edible roots.
[d]Includes raw sugar; excludes syrups and honey.
[e]Includes cocoa beans.
[f]Fresh equivalent.
[g]Includes offal, poultry and game expressed as carcass weight, excluding slaughter fats.
[h]Fresh egg equivalent.
[i]Landed weight.
[j]Excludes butter; includes milk products as fresh milk equivalent.
[k]Pure fat content.

TABLE 4-7

Summary of Per Capita Estimates of World Food Consumption[a]

Food Groups	Worldwide			
	Minimum (g/day)	Maximum (g/day)	Minimum (kg/yr)	Maximum (kg/yr)
Cereals[b]	185	446	67.5	162.8
Starchy roots[c]	44	473	16.1	172.6
Sugar[d]	22	135	8.0	49.3
Pulses and nuts[e]	11	56	4.0	20.4
Vegetables and fruits[f]	128	516	46.7	188.3
Meats[g]	24	312	8.8	113.9
Eggs[h]	3	55	1.1	20.1
Fish[i]	12	38	4.4	13.9
Milk[j]	51	850	18.6	310.2
Fats and oils[k]	9	56	3.3	20.4

[a]Source: Adapted from ICRP, 1975

[b]Flour and milled

[c]Includes sweet potatoes, cassava and other edible roots.

[d]Includes raw sugar; excludes syrups and honey.

[e]Includes cocoa beans.

[f]Fresh equivalent.

[g]Includes offal, poultry and game expressed as carcass weight, excluding slaughter fats.

[h]Fresh egg equivalent.

[i]Landed weight.

[j]Excludes butter; includes milk products as fresh milk equivalent.

[k]Pure fat content.

reported in Table 4-5. Food consumption per capita is reported by geographical regions in Table 4-6; the worldwide profile is presented in Table 4-7. The data in these tables summarize human fluid, respiratory and food intake. NAS combined these intake data with information on carbon tetrachloride contamination of water, air and food to estimate human exposure to carbon tetrachloride. A summary of the NAS computations of carbon tetrachloride intake follows.

4.4.1. **Water.** The potential carbon tetrachloride uptake from fluids is shown in Table 4-8. NAS used in its calculations the levels of carbon tetrachloride reported in drinking water in the NORS study (Symons et al., 1975). These levels were <0.003 mg/ℓ. In a later report (the NOMS) carbon tetrachloride was detected at levels of 0.0024 to 0.0064 mg/ℓ in U.S. drinking water (U.S. EPA, 1980a). Estimation of carbon tetrachloride consumption derived by using the NOMS maximum value of 0.0064 mg/ℓ were included in the data in Table 4-8. These data were calculated by multiplying the minimum and maximum concentrations in drinking water by the minimum, maximum and "reference" consumption of drinking water. These calculations were based upon the following assumptions:

1. 100% of the carbon tetrachloride ingested is absorbed.
2. Commercially-produced drinks and drinks reconstituted in the home contain the same level of carbon tetrachloride as does drinking water.
3. All fluids contain the indicated carbon tetrachloride concentration.

Values for total fluid uptake include drinks such as milk, not prepared by mixing with water.

4.4.2. **Air.** The potential carbon tetrachloride uptake from air is presented in Table 4-9. The minimum level for carbon tetrachloride in air used

TABLE 4-8

Carbon Tetrachloride Uptake from Fluids (mg/yr) Calculated by Assuming 100% Absorption[a]

Exposure	Fluid	Adult Man, Fluid Intake			Adult Woman, Fluid Intake			Child, Fluid Intake		
								5-14 yr		10 yr
		Minimum	Maximum	Reference	Minimum	Maximum	Reference	Minimum	Maximum	Reference
Minimum (0.002 mg/ℓ)[c]	Tap water	0.03	0.53	0.11	0.03	0.53	0.07		0.58	0.15
	Other[b]	0.23	1.06	1.10	0.23	1.06	0.80			0.55
	Total fluid	0.73	2.70	1.42	0.73	2.70	1.02	0.73	1.22	1.02
Maximum (0.003 mg/ℓ)[c]	Tap water	0.05	0.80	0.16	0.05	0.80	0.11	0.59	0.86	0.22
	Other[b]	0.35	1.59	1.64	0.35	1.59	1.20			0.82
	Total fluid	1.10	4.05	2.14	1.10	4.05	1.53	1.10	1.83	1.53
Maximum (0.0064 mg/ℓ)[d]	Tap water	0.10	1.70	0.35	0.10	1.70	0.23	1.26	1.84	0.47
	Other[b]	0.75	3.39	3.50	0.75	3.39	2.57			1.75
	Total fluid	2.34	8.65	4.56	2.34	8.65	3.27	2.34	3.90	3.27

[a]Adapted from NAS, 1978

[b]Includes water-based drinks such as tea, coffee, soft drinks, beer, cider, wine.

[c]Calculated by multiplying the exposure concentration (mg/ℓ) x fluid intake (ℓ/yr) (from Table 4-3 for minimum and maximum intakes and Table 4-4 for reference intakes).

[d]Similar calculations based upon NOMS maximum drinking water levels of 6.4 μg/ℓ.

TABLE 4-9

Estimated Human Uptake of Carbon Tetrachloride from the Outdoor Atmosphere[a,b]
(mg/yr)

	Adult Man, Exposure			Adult Woman, Exposure		
	Minimum	Typical	Maximum	Minimum	Typical	Maximum
Average inhaled[c]	6.3	7.9	951	5.8	7.3	872
Minimum absorbed[d]	3.6	4.5	542	3.3	4.1	457
Maximum absorbed[d]	4.1	5.2	618	3.8	4.7	567
	Child, 10 yr, Exposure			Infant, 1 yr, Exposure		
Average inhaled[c]	4.2	5.2	623	1.1	1.3	159
Minimum absorbed[d]	2.4	3.0	355	0.6	0.6	90.0
Maximum absorbed[d]	2.7	3.4	405	0.7	0.9	103

[a]Source: Adapted from NAS, 1978

[b]The absorption range used is that reported by Lehmann and Schmidt-Kehl (1936). Based upon human data, the range given is 57 to 65%, calculated from the concentration of carbon tetrachloride in the inhaled air and that found in the exhaled breath.

[c]Calculated by applying respiratory volume per year (see Table 4-5) to the general range of atmospheric concentrations.

[d]Calculated by applying the range of percent absorptions to the total amount inhaled per year.

by NAS was 0.00075 mg/m^3, the typical level was 0.00094 mg/m^3 and the maximum level was 0.113 mg/m^3, reported by Lillian et al. (1975) for a monitoring sample for Bayonne, New Jersey. These values were multiplied by the average quantity of air inhaled per year (from Table 4-5) to arrive at the average quantity of carbon tetrachloride inhaled per year. In the NAS report, this quantity was then multiplied by the minimum and maximum values of the absorption range for carbon tetrachloride in air as reported for humans by Lehmann and Schmidt-Kehl (1936). These calculations resulted in values for the minimum and maximum yearly absorption of carbon tetrachloride from air. The issue of percent absorption of CCl_4 has not been clearly defined. As discussed in Section 7.1, the percent absorbed may vary between species. The range reported by Lehmann and Schmidt-Kehl and used in the NAS report, is retained here since it is the only human data available. See Section 7.1 for a more detailed discussion on absorption of CCl_4.

4.4.3. **Food.** The potential carbon tetrachloride uptake from foods is presented in Table 4-10. Values of carbon tetrachloride found in foods and used in these calculations are presented in Table 4-11. To arrive at uptake values, the minimum and maximum levels of carbon tetrachloride in foods were multiplied by the minimum and maximum worldwide food intake for individuals for each food category. NAS calculated a per capita uptake of 0.21 to 7.33 mg carbon tetrachloride from food each year.

The NAS absorption calculations did not include the yearly uptake through the consumption of bread. The average consumption of carbon tetrachloride attributed to bread has been calculated elsewhere as 777 ng/day or 0.3 mg/yr (45 FR 68534-68584). The data on consumption in bread are included in the summary of carbon tetrachloride uptake from all sources (Table 4-12).

TABLE 4-10

Carbon Tetrachloride Uptake from Food Supplies (mg/yr), Calculated by Assuming 100% Absorption[a,b]

Food Group[c]	Worldwide Food Intake[d]	Exposure		
		Minimum	Average	Maximum
Milk products	Minimum	0.004	--	0.26
	Maximum	0.06	--	4.34
Eggs	Minimum	--	0.001	--
	Maximum	--	0.01	--
Meats	Minimum	0.06	--	0.08
	Maximum	0.80	--	1.02
Fats and oils	Minimum	0.002	--	0.06
	Maximum	0.01	--	0.37
Vegetables and fruits	Minimum	0.14	--	0.37
	Maximum	0.56	--	1.51
Fish and seafood	Minimum	<0.001	--	0.03
	Maximum	0.001	--	0.08
Total, all food supplies	Minimum	0.21	--	--
	Average	--	1.12	--
	Maximum	--	--	7.33

[a]Source: Adapted from NAS, 1978

[b]Calculated by applying worldwide food intake ranges for various food groups (see Table 4-7) to the range of concentrations found in various foods (see Table 4-10).

[c]As used in Tables 4-6 and 4-7.

[d]From Table 4-7.

TABLE 4-11

Summary of Carbon Tetrachloride Concentrations in Food Supplies*
(ppb by weight, µg/kg)

Food Group	Carbon Tetrachloride		
	Minimum	Average	Maximum
Milk products	0.2	--	14.0
Eggs	--	0.5	--
Meats	7.0	--	9.0
Fats and oils	0.7	--	18.0
Vegetables and fruits	3.0	--	8.0
Fish and seafood	0.1	--	6.0

*Source: Adapted from McConnell et al., 1975; Pearson and McConnell, 1975

TABLE 4-12

Relative Uptake of Carbon Tetrachloride from the Environment by Adult Male[a]

Source	Minimum Exposure[b] (mg/yr)	Minimum Exposure[b] (% of total)	Typical Exposure[c] (mg/yr)	Typical Exposure[c] (% of total)	Maximum Exposure[d] (mg/yr)	Maximum Exposure[d] (% of total)
Fluid intake	0.73	16	3.13	34	8.65	1
Atmosphere	3.60	79	4.75	51	618	98
Food supply	0.21	5	1.42	15	7.63	1
Total	4.54	--	9.30	--	634.28	

[a]Source: Adapted from NAS, 1978

[b]For minimum conditions: assume minimum intake for fluids, minimum absorption from atmosphere, and minimum intake for food supplies.

[c]For typical conditions: assume reference man intake for fluids, 0.0044 mg/ℓ as average of concentrations in NOMS study; for intake from atmosphere, assume average of minimum and maximum absorption rates for typical exposure; for intake from food supplies, assume average exposure and intake from NAS, 1978 plus 0.3 mg/yr from bread consumption (45 FR 68534-68584).

[d]For maximum conditions: assume maximum intake for fluids, carbon tetrachloride at 0.0064 mg/ℓ as in NORS study; maximum absorption from atmosphere; and maximum intake from food from NAS, 1978 plus 0.3 mg/yr from bread consumption (45 FR 68534-68584).

4.4.4. **Soil.** The potential uptake of carbon tetrachloride from soil is unknown. This includes agricultural runoff as well as uptake from plants.

4.5. SUMMARY

Carbon tetrachloride is produced commercially from the chlorination of methane, propane, ethane propylene and carbon disulfide. Production has declined over the last 10 years and a 1.0%/year decline is projected through 1985.

CCl_4 is ubiquitous in the environment and has been detected in concentrations of generally <.01 mg/ℓ in water, <.01 mg/m^3 in air and <.01 mg/kg in food. Higher levels of carbon tetrachloride have been detected in urban air, and in grain and food products made from grain. Increased levels of carbon tetrachloride in urban air are probably due to the industrial use of carbon tetrachloride and products containing carbon tetrachloride. The presence of carbon tetrachloride in grains or grain products is due in part to the use of fumigants containing carbon tetrachloride.

Data on the uptake of carbon tetrachloride by the adult male are summarized in Table 4-12. At minimum exposure levels, uptake from the air appears to be the major source of exposure (79%), followed by fluids (16%) and the food supply (5%). At typical exposure levels, uptakes from fluid (34%) and food (15%) appear to increase with respect to that from air (51%). At maximum exposure levels, 98% of carbon tetrachloride uptake is estimated to be from air. Amount of uptake from soil is unknown.

All of the carbon tetrachloride in the environment can be accounted for by anthropogenic activities. Carbon tetrachloride, unlike chloroform and other halocarbons, does not appear to be indirectly formed during water chlorination. No natural sources of carbon tetrachloride are known.

5. FATE AND TRANSPORT

5.1. FATE

5.1.1. Water. Carbon tetrachloride tends to evaporate from dilute aqueous solutions, with a half-life of only 29 minutes (Dilling et al., 1975). Chemical stability in aquatic environments is high, with half-lives of $7 \times 10^3/C$ years, where C is concentration in mg/ℓ. This formula is derived from the second order rate constant of $4.8 \times 10^{-7}/mol^{-1}/sec^{-1}$ (Mabey and Mill, 1978). Singh et al. (1976) give an approximate hydrolytic half-life for carbon tetrachloride in oceans as 7×10^4 years. This figure is lower than that predicted using measured ocean concentrations (Pearson and McConnell, 1975) and the above second order formula. For the maximum Atlantic Ocean concentration of 2.4×10^{-6} mg/ℓ, the half-life is 2.9×10^6 years. In any case, carbon tetrachloride is extremely stable in water, with losses primarily due to other factors such as evaporation, sediment adsorption and organism uptake. Singh et al. (1976) estimate that 2 to 3% of all biospheric carbon tetrachloride is in solution. Although carbon tetrachloride is not easily transported to ground water due to its high volatility, low solubility and low mobility in soil, any contamination is likely to persist for several years and accumulate.

5.1.2. Air. Chemical stability, a long lifetime, and uniform mixing characterize the fate of carbon tetrachloride in air. Mixing is assumed to be uniform to approximately 18 km elevation, with a pseudo-first order removal rate of 1×10^{-3} yr^{-1}, mainly via gas-phase reactions involving the electronic state $O(^1D)$ (Galbally, 1976). The principal sink is considered to be the stratosphere (Galbally, 1976; Singh et al., 1976), with significant loss of carbon tetrachloride limited by the rate of transport from the troposphere. Above 18 km, Galbally estimates that stratospheric

photolysis is dominant, with a pseudo-first order rate constant of 1.7×10^{-2} yr^{-1}. This photodissociation is attributed to UV radiation mainly in the 195 to 225 nm region. Galbally estimates 50% errors in each of the above rate constants.

UV photolysis was also shown experimentally by Lillian et al. (1975) using a 1 ℓ Hanovia photochemical reactor with zero air and a high pressure mercury vapor lamp (0.034 W/cm^2 in the 220 to 230 nm region). Simulated tropospheric irradiation produced no discernable dissociation of carbon tetrachloride in NO_2-air mixtures. The lifetime (time to reach 1/e of initial concentration) of carbon tetrachloride in the stratosphere is estimated at 53 hours (Lillian et al., 1975). The tropospheric lifetime has been estimated at 330 years (Singh et al., 1976). The overall atmospheric lifetime of carbon tetrachloride is estimated at 60 to 100 years by Singh et al. (1976) and "several decades" by Galbally (1976).

Carbon tetrachloride in the stratosphere is considered to be a potential source of chlorine via photodissociation which may, on a molecule-by-molecule basis, have the potential approximately as strong as CFC-11 and CFC-12 to catalyze the destruction of the ozone layer (Hanst, 1978; NAS, 1978). The proposed mechanisms and the potential for destruction of ozone are tentative due to the paucity of data on stratospheric carbon tetrachloride and to the uncertainties in the structure and evaluation of the transport models (Sugdon and West, 1980).

5.1.3. Soil. Pertinent information concerning the degradation or transformation of carbon tetrachloride in soil could not be located in the available literature. Galbally (1976) calculates that carbon tetrachloride in all land biota is probably <0.2% of that in the atmosphere and concludes that land biota are an insignificant sink for carbon tetrachloride.

5.2. TRANSPORT

5.2.1. Water. Carbon tetrachloride introduced into water resources will be transported by movement of surface and ground water, and by evaporation into the atmosphere. Studies in N.W. England show transfer of carbon tetrachloride from the atmosphere to surface water due to heavy rainfall (McConnell, 1976). In dilute aqueous solutions, the evaporative half-life is 29 minutes (Dilling et al., 1975). Large, concentrated quantities, however, tend to remain as one mass or "slug". A spill into the Ohio River of approximately 70 tons of carbon tetrachloride produced a slug that was estimated to be 74 miles long (U.S. Congress, 1977). Degradation of carbon tetrachloride by hydrolytic reactions is slow; the approximate half-life is 7000 years (Mabey and Mill, 1978). Thus, volatilization is considered to be the main process for removing carbon tetrachloride from aquatic systems.

5.2.2. Air. The high vapor pressure, low water solubility and high specific gravity of carbon tetrachloride influence its disposition in air environments. Volatilization to the atmosphere from land and water should be rapid. Actual transport in the air, however, has been infrequently demonstrated. Penkett et al. (1979) found higher atmospheric values over Great Britain during easterly winds than during westerly winds, reflecting the difference in anthropogenic activity under the two trajectories. Other reports show minor global variation in concentration of carbon tetrachloride (Singh et al., 1976; Lovelock et al., 1973). Vertical transport from the lower atmosphere to the stratosphere is estimated at roughly 25%, assuming an overall atmospheric lifetime of 75 years and a quasi-steady state loading (Singh et al., 1976). Neely (1977) modeled the transport of carbon tetrachloride vertically from the ocean through the troposphere to the stratosphere, and horizontally between northern and southern hemispheres. Trans-

fer between hemispheres was considered substantial, with a first-order rate constant (in each direction) of 0.9 yr^{-1}. Neely's simulation results agreed well (within 20%) with observed concentrations.

5.2.3. Soil. There is little quantitative information about adsorption of carbon tetrachloride onto sediments. Following Briggs (1973), the sorption potential can be estimated by the following formula:

$$\log Q = 0.524 \log P + 0.618$$

where P is the octanol/water partition coefficient and Q is the organic matter/water partition coefficient. For carbon tetrachloride, log P = 2.64, and thus Q = 100.3. This value suggests that carbon tetrachloride has "low" mobility in soil, as defined by Briggs (1973), indicating that it can move with soil, sediment or particulate matter. Morris and Johnson (1976) state that periods of high agricultural runoff (river turbidity) are directly correlated with high chloroform concentration in finished water and imply that similar associations hold for carbon tetrachloride and haloforms in general. Statistical analysis of their data, however, shows poor linear correlation (r = 0.06) between river turbidity and carbon tetrachloride levels, with strong peaks occurring during periods of both low and high turbidity. Pearson and McConnell (1975) investigated chlorinated hydrocarbons in marine environments, but could not show any significant relationships between levels in sediment and levels in overlying seawater, sediment particle size or geographic features. More research is clearly needed on transport via rainfall, directly from the atmosphere and indirectly through agricultural runoff, in order to assess the importance of soil for transport of carbon tetrachloride.

5.3. BIOACCUMULATION/BIOCONCENTRATION

The data of Kopperman et al. (1976) suggest that polar organochlorine compounds are easily biodegraded, while non-polar, highly lipophilic compounds accumulate. Bioaccumulation is directly related to the octanol/water partition coefficient (P) of the compound (Neely et al., 1974). The log octanol/water partition coefficient (log P) of carbon tetrachloride is 2.64 (Leo et al., 1971; Neely et al., 1974; Chiou et al., 1977), indicating a possible tendency for this compound to bioaccumulate under conditions of constant exposure. However, although carbon tetrachloride and other organochlorines are lipophilic and tend to concentrate in fatty tissues, there is no evidence that they magnify through the food chain (Pearson and McConnell, 1975). Difficulties in the Pearson and McConnell (1975) study discourage estimates of bioaccumulation based on their results. Barrows et al. (1980) reported a carbon tetrachloride bioconcentration factor (BCF = tissue concentration divided by water concentration) of 30 for bluegill sunfish (Lepomis macrochirus) and a tissue half-life of <1 day. The authors stated that the short tissue half-life makes it unlikely that carbon tetrachloride will biomagnify in fish unless exposure is continuous or prolonged. Neely et al. (1974) estimated the steady state BCF for rainbow trout (Salmo gairdneri) to be 17. Data could not be found for bioconcentration of carbon tetrachloride in shellfish.

5.4. SUMMARY

Transport and fate of carbon tetrachloride have been studied most extensively in air, with some information on water levels and almost no data on soil. Carbon tetrachloride is extremely stable in water, the lower atmosphere and the troposphere. Photodissociation in the stratosphere is rapid.

Global distribution of carbon tetrachloride in air is nearly uniform. Bioconcentration in fish is low. Research needs are most pronounced for fate in soil, transport to and fate in the stratosphere, and bioaccumulation/bioconcentration in shellfish.

6. ECOLOGICAL EFFECTS

Data on the ecological effects of carbon tetrachloride are somewhat limited. The reasons for this can be inferred from the physical and chemical properties of the compound as discussed in Chapter 3. Carbon tetrachloride is quite volatile and so does not readily accumulate in either terrestrial or aquatic environments and is rapidly diluted to low concentrations in the troposphere. While major spills of carbon tetrachloride have occurred, ecosystem effects have not been documented or have not been considered to be so significant as to merit further investigation (NAS, 1978). Apparent acute effects have been minimal and, consequently, chronic effects on fish and wildlife associated with long-term exposures at low levels are unlikely.

6.1. EFFECTS ON NON-TARGET ORGANISMS

Because carbon tetrachloride is used as a pesticide, primarily as a grain and soil fumigant, non-target soil organisms are undoubtedly affected (NAS, 1978). The interaction of carbon tetrachloride with anaerobic organisms has been studied by Wood et al. (1968) using extracts of Methanobacillus omelianskii and the N^5-methyl tetrahydrofolate-homocysteine transmethylase of Escherichia coli B. The authors found that low concentrations of carbon tetrachloride inhibit cobamide-dependent methyl transfer reactions in these cell extracts. The possibility that carbon tetrachloride might inhibit methyl transfer systems of microbial organisms in nature should be investigated, although there is no imminent hazard (NAS, 1978).

6.1.1. Aquatic Life Toxicology. The majority of the acute toxicity data for carbon tetrachloride and aquatic organisms has been determined using static procedures with unmeasured test concentrations. Results of these tests may underestimate the acute toxicity of carbon tetrachloride due to its volatility. No acute or chronic effects were observed at a concentration lower than 3400 µg/ℓ.

6.1.2. Acute Toxicity. The 48-hour EC_{50} is 35,200 µg/ℓ for Daphnia magna (Table 6-1). The bluegill has been tested (Dawson et al., 1977; U.S. EPA, 1978) and the 96-hour LC_{50} values are 125,000 and 27,300 µg/ℓ, respectively (see Table 6-1). The reason for this large difference is not clear, but may have been caused by the volatility of this compound. There appears to be no great difference in sensitivity between the two tested species. A flow-through test result for the fathead minnow is 43,100 µg/ℓ. However, no comment can be made concerning the effect of test conditions on test results.

Carbon tetrachloride (1 mℓ/kg, i.p.) produced a 5- to 10-fold increase in serum GOT, GPT and ICD enzyme activities in rainbow trout (Salmo gairdneri), whereas exposure of trout to CCl_4 in tank water (1 to 80 mg/ℓ) produced neither mortality nor changes in serum enzyme activities (Statham et al., 1978). Tissue residue analysis revealed the highest concentration of CCl_4 in the adipose tissue followed by levels in the liver, brain, spleen and gills irrespective of the administration route. Elimination rates of ^{14}C residues occurred at 2 hours for both i.p. and ambient water exposure routes and were 4.8 and 0.75 µmol/g, respectively. Histologic examination of tissues revealed varying degrees of liver and splenic neurosis 6 hours following administration of CCl_4 (Statham et al., 1978).

A single i.p. injection of CCl_4 resulted in a significant decrease in total plasma protein concentration of rainbow trout at 12, 24 and 36 hours post-treatment (Pfeifer and Weber, 1981). Plasma albumen was also reduced significantly at the 2 mℓ/kg dose level. The authors suggested that several factors, including intestinal inflammation and hemorrhage, may have contributed to the observed effects on plasma protein concentration. In an earlier study (Pfeifer and Weber, 1980) using the same species, it was

TABLE 6-1

Acute Values for Carbon Tetrachloride

Species	Method*	LC_{50}/EC_{50} (µg/ℓ)	Species Acute Value (µg/ℓ)	Reference
		FRESHWATER SPECIES		
Cladoceran, *Daphnia magna*	S, U	35,200	35,200	U.S. EPA, 1978
Fathead minnow, *Pimephales promelas*	FT, M	43,100	43,100	Kimball, manuscript
Bluegill, *Lepomis macrochirus*	S, U	125,000	-	Dawson et al., 1977
Bluegill, *Lepomis macrochirus*	S, U	27,300	58,000	U.S. EPA, 1978
		SALTWATER SPECIES		
Tidewater silversides, *Menidia beryllina*	S, U	150,000	150,000	Dawson et al., 1977

*S = static, FT = flow-through, U = unmeasured, M = measured

No Final Acute Values are calculable since the minimum data base requirements are not met.

determined that a single i.p. dose of CCl_4 (2.0 mℓ/kg) produced either oliguria or anuria as early as 1 hour post-treatment. A significant reduction in urine flow as well as a significant increase in relative wet body weight were noted. Histological examination of kidney tissue at 24 hours post-treatment revealed early localized pathological changes, although extensive morphological damage was not evident. This, in conjunction with the early oliguria, suggests that the observed reduction in urine flow rate was not due to a direct toxic effect on the kidney (Pfeifer and Weber, 1980).

Morphological changes in the liver of two species of carp (Cyprino carpio and Carassius auratus) were observed following i.p. injection of CCl_4 at 0.3 to 5.0 mℓ/kg for at least 8 days (Jiang and Zhang, 1979). The following changes were noted in the liver tissue of both species: (1) proliferation in number of vacuoles, (2) nuclei enlargement in some cells, and (3) contraction and deformation of the nuclear membrane. These findings are in accord with those of studies discussed previously that reported decreased plasma protein concentrations and, consequently, reflect the hepatotoxicity of CCl_4.

Only two saltwater fish and no invertebrate species have been tested. The 96-hour LC_{50} for the tidewater silversides (Menidia beryllina) is 150,000 µg/ℓ (see Table 6-1). The other datum is an estimated 96-hour LC_{50} for the dab (Limanda limanda) of about 50,000 µg/ℓ.

6.1.3. Chronic Toxicity. No chronic test has been conducted with a freshwater invertebrate species or any saltwater species. An embryo-larval test with the fathead minnow (Pimephales promelas) was conducted, and no adverse effect was observed at carbon tetrachloride concentrations up to 3400 µg/ℓ (U.S. EPA, 1978).

6.2. TISSUE RESIDUES

The bluegill (Lepomis macrochirus) bioconcentrated carbon tetrachloride at equilibrium to a factor of 30 times within 21 days. The biological half-life in these tissues was less than 1 day. In addition, Neely et al. (1974) exposed the rainbow trout to carbon tetrachloride and estimated a steady-state BCF of 17. Similarly, Barrows et al. (1978) determined a BCF for bluegill sunfish of 30. These results indicate that tissue residues of carbon tetrachloride would not pose a potential environmental hazard to aquatic life.

6.3. INDIRECT ECOSYSTEM EFFECTS

Indirect ecosystem effects of carbon tetrachloride (e.g., those caused by enhanced UV-B radiation) are similar to those of other halocarbons and result from photodissociation of the compound and subsequent destruction of stratospheric ozone (NAS, 1978, 1982). For more discussion of these effects, see Chapters 5 and 11.

6.3.1. Effect on Stratospheric Ozone. Carbon tetrachloride may contribute to an overall reduction in stratospheric ozone. Following a relatively rapid tropospheric mixing with other halogenated methanes, there is a relatively slow entry of CCl_4 into the stratosphere, followed by a random ascent to altitudes (in excess of 25 km) where solar ultraviolet (UV) radiation in the range 185 to 225 nm photodissociates CCl_4 resulting in the generation of "odd chlorine" (i.e., a variety of chlorine-containing species) (NAS, 1978). Dependent upon the rates of a variety of chemical reactions and the rate of odd chlorine to the troposphere, each odd chlorine is responsible for the destruction of several thousand ozone molecules. It has been estimated that CCl_4, along with hydrochloric acid (HCl) and methyl chloride (CH_3Cl), contributes <1% of the total reduction in strato-

spheric ozone, comparable to the rate of ozone reduction attributed to chlorofluoromethanes. The relative importance of HCl and CH_3Cl is reduced, however, due to atmospheric "rainout" and degradative processes, respectively (NAS, 1978).

6.3.2. Effect on UV Flux. As indicated above, changes in stratospheric ozone content result in alterations of UV flux to the earth's surface. This involves the biologically damaging wavelengths in the 290 to 320 nm range (UV-B regions). The molecular basis of the effects of these wavelengths is the alteration of protein and nucleic acid structures impacting both genetic replication and protein synthesis mechanisms (NAS, 1978). While there exist molecular repair processes that mitigate this damage, they are not totally effective.

Increased UV-B radiation adversely affects a variety of plant species in terms of depressed photosynthetic activity and reduced growth rate. Studies of both agriculturally significant plants and higher non-agricultural species (cited by NAS, 1978) demonstrate a diverse response to enriched levels of UV radiation. These findings include, in addition to depressed photosynthetic activity, inhibition of seed germination and increased somatic mutation rates. However, adaptation of these plant species appears to be sufficient under current ambient levels to maintain food crop yields (NAS, 1982). Although effects on natural ecosystems are difficult to predict, the ecological impact of such effects may be significant to natural communities taken collectively, even if only a few constituent species are affected. The magnitude of such effects cannot be predicted since natural populations have adapted to current ambient levels to maximum reproduction potential

(NAS, 1982). In addition to the direct effect of UV-B wavelengths on these species, effects on interacting and/or dependent species must be considered (NAS, 1978) in assessing ecosystem effects.

6.4. SUMMARY

Only two freshwater fish and one invertebrate species have been acutely tested and a 96-hour LC_{50} has been determined as low as 27,300 µg/ℓ. No definitive chronic data are available. Tissue residues of carbon tetrachloride do not appear to be a problem since available data suggest a BCF of <30.

The available data for carbon tetrachloride indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 35,200 µg/ℓ and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive freshwater aquatic life.

The available data for carbon tetrachloride indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 50,000 µg/ℓ and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of carbon tetrachloride to sensitive saltwater aquatic life.

Indirect effects of CCl_4 are associated with reduced levels of atmospheric ozone and concomitant increases of UV-B radiation flux. There are known differences in species sensitivities to increases in UV-B radiation. Laboratory studies have identified effects associated with such increases. However, it appears that in the natural ecosystem, some plants and animals have adapted to current ambient levels. Thus, the magnitude of effects of enhanced UV-B radiation cannot be predicted either collectively or for individual species in the natural environment.

7. COMPOUND DISPOSITION AND RELEVANT PHARMACOKINETICS

This chapter is divided into four sections: absorption, distribution, metabolism and excretion of carbon tetrachloride. The absorption section begins with a discussion of the chemical's partition coefficients. As would be expected for a compound with high partition coefficients for oil/air and oil/water, carbon tetrachloride is reported to be absorbed readily through the lungs, and also through the intestinal tract and skin. Once absorbed, the chemical and its metabolites are reported to be distributed widely throughout the body, with high concentrations in liver, bone marrow, blood, muscle, fat and brain. Several metabolites of CCl_4 have been identified, including chloroform, hexachloroethane and carbon dioxide. Carbonyl chloride has been postulated as a metabolite by inference based on an *in vitro* study of a [^{14}C]carbon tetrachloride incubation system. CCl_4 metabolism has been proposed to involve a complex with ferrocytochrome heme and formation of the free radical CCl_3. Carbon tetrachloride and its metabolites are reported to be excreted principally in exhaled air, but also in urine and feces.

7.1. ABSORPTION

7.1.1. Partition Coefficients. Partition coefficients for various chlorinated solvents, including carbon tetrachloride, were determined by several experiments (Morgan et al., 1972; Sato and Nakajima, 1979; Powell, 1945). The partition coefficient is a measure of the relative solubility of a substance in two media. The oil/air and oil/water partition coefficients can be used as indicators of solubility in lipids. The values of these and other partition coefficients for carbon tetrachloride, listed in Table 7-1, show this chemical to be lipophilic. Because of its lipophilic nature, one

TABLE 7-1

Partition Coefficients for Carbon Tetrachloride*

Parameter	Partition Coefficients 20°C	25°C	37°C
Olive oil/air	--	142	361
Blood serum/air	--	6	--
Blood/air	3.6-5.2	--	2.4
Water/air	--	0.25	--
Olive oil/water	--	1440	--
Olive oil/serum	--	23	--
Olive oil/blood	--	--	150

*Source: Adapted from Morgan et al., 1972; Sato and Nakajima, 1979; Powell, 1945

would predict that carbon tetrachloride could be absorbed by ingestion, inhalation and skin contact. This prediction is borne out by results of the experimental studies described below.

7.1.2. Absorption from the Gastrointestinal Tract. Absorption of carbon tetrachloride from the gastrointestinal tract of dogs was studied by Robbins (1929). In a series of experiments, the author determined the amount of carbon tetrachloride absorbed from the ligated stomach, small intestine and colon by measuring the concentration of carbon tetrachloride exhaled. The greatest concentration of carbon tetrachloride in exhaled air was seen after injection of the chemical into the small intestine. Direct injection of carbon tetrachloride into the colon resulted in a lower concentration of the chemical in exhaled air. After introduction directly into the stomach by intubation, no carbon tetrachloride was detected in exhaled air. The method of detection in these experiments was thermal conductivity, with stated detection limits of 1 part in 10 by volume of expired air. Thus, the results of the experiment can be viewed as a qualitative indication of relative absorption from the various components of the gastrointestinal tract, rather than as quantitatively accurate results.

The enhancement in the extent of carbon tetrachloride absorption with ingestion of fat or alcohol has been reported (Nielsen and Larsen, 1965; Robbins, 1929; Moon, 1950). It also appears that the absorption of carbon tetrachloride from the gastrointestinal tract may vary with different species because it occurs more quickly in rabbits than in dogs (Lamson, 1923).

Marchland et al. (1970) studied the effect of SKF 525A on the distribution of an oral carbon tetrachloride dose on rats. Control animals exposed only to carbon tetrachloride were found to excrete at least 80% of the orally administered dose within 10 hours via the lungs. This indicates that at least 80% of the carbon tetrachloride dose was absorbed orally.

Stokinger and Woodward (1958) report an "accurate" absorption factor of 50% via ingestion, however, they do not reference their conclusion.

7.1.3. Absorption by Inhalation. In 1950, von Oettingen et al. studied the absorption of carbon tetrachloride by inhalation in beagle dogs. The sex was unspecified, but the authors stated that at least five dogs were used in each experiment. The dogs inhaled carbon tetrachloride (purity unspecified) at a concentration of 94,500 mg/m^3 for 475 minutes through a two-way valve attached to the cannulated trachea. Blood samples were taken at unspecified intervals and analyzed for carbon tetrachloride. Data presented graphically showed that the concentration of carbon tetrachloride in blood reached a maximum of 31.2 to 34.3 mg/100 cc (0.20 to 0.22 millimole %) after ≈300 minutes of exposure and remained at that level for the duration of the exposure.

McCollister et al. (1951) investigated the absorption of carbon tetrachloride by inhalation using Rhesus monkeys. Three female monkeys inhaled 99.9% ^{14}C-labeled carbon tetrachloride vapor at an average concentration of 290 mg/m^3 for 139, 344 or 300 minutes, respectively. The authors calculated by difference between inhaled and exhaled air that the monkeys absorbed an average of 30.4% of the total amount of carbon tetrachloride inhaled. Analysis of blood drawn after 270 minutes of exposure showed that the ^{14}C radioactivity was equal to 0.297 mg carbon tetrachloride/100 g of blood, distributed as follows: 56.2% as carbon tetrachloride, 16.5% as "acid-volatile" carbonates and 27.3% as nonvolatile material. No attempt was made to characterize metabolites in this study. The radioactivity no longer associated with the carbon tetrachloride was described by the stage in the analytical procedure where it was found.

Stokinger and Woodward (1958) report an "accurate" absorption factor of 30% via inhalation, however, they do not reference their conclusion. Lehmann and Schmidt-Kehl (1936) studied humans exposed to CCl_4 via inhalation. In separate experiments, individuals (number unknown) were enclosed in a room of CCl_4 vapors for varying amounts of time. The amount of CCl_4 available for inhalation was measured. The percent absorbed was calculated from the concentration of CCl_4 in the inhaled air minus the amount found in the exhaled breath. The reported range of percent absorption was 57 to 65%.

7.1.4. Absorption Through the Skin. McCollister et al. (1951) exposed the skins of one male and one female Rhesus monkey to ^{14}C-labeled carbon tetrachloride vapor. To determine the amount of absorption, blood and exhaled air were analyzed for ^{14}C radioactivity. After a skin exposure of 240 minutes to carbon tetrachloride vapor at 3056 mg/m^3, the blood of the female monkey contained carbon tetrachloride at 0.012 mg/100 g and the exhaled air contained 0.0008 mg/ℓ. After exposure to 7345 mg/m^3 for 270 minutes, blood of the male monkey contained carbon tetrachloride at 0.03 mg/100 g and the exhaled air contained 0.003 mg/ℓ.

Three human volunteers, sex unspecified, immersed their thumbs in carbon tetrachloride for 30 minutes in an experiment to measure skin absorption (Stewart and Dodd, 1964). The carbon tetrachloride was analyzed by infrared spectroscopy and was found to contain no detectable impurities. The concentration of carbon tetrachloride in alveolar air was used as the indicator of absorption and was measured at 10, 20 and 30 minutes after the start of exposure and at 10, 30, 60, 120 and 300 minutes after cessation of exposure. Carbon tetrachloride was present in the alveolar air at each time interval, reached a maximum concentration range of 2.8 to 5.7 mg/m^3 30

minutes after exposure, and decreased exponentially thereafter. The authors concluded that carbon tetrachloride could be absorbed by the skin in toxic amounts if the chemical came in contact with arms and hands.

7.2. DISTRIBUTION

The distribution of carbon tetrachloride in humans, presumably after chronic low level exposure through various media (food, water and air), has been reported by McConnell et al. (1975). Measurements were made from post-mortem tissues of several individuals; arithmetic means (in mg/kg) were about: body fat, .008 (range .002 to .024); liver, .003 (range .001 to .005); and kidney, .002 (range .001 to .003). Unfortunately these values represent possible concurrent trichloroethane concentrations.

Robbins (1929) administered 159 g (100 cc) carbon tetrachloride, purity unspecified, to three anesthetized dogs by stomach tube. The dogs were sacrificed at 6, 23 and 24 hours after treatment. Blood and various tissues were analyzed for carbon tetrachloride by converting the organic chloride to inorganic chloride and titrating the inorganic chloride by the Volhard method, which is accurate to 0.1 to 0.2%. The results of the blood and tissue analysis are shown in Table 7-2. Note that the uptake of CCl_4 was probably reduced because of probable reduced G-I (portal) circulation during anesthesia (Withey, 1981).

From the experimental data, it appears the limit of detection was in the range of 4 to 5 mg carbon tetrachloride/100 g of tissue. In addition, it appears that the liver, bone marrow, blood and muscle retained the most carbon tetrachloride for the longest time.

In 1950, von Oettingen et al. reported the tissue distribution of carbon tetrachloride in beagle dogs, each weighing ≈10 kg, exposed to carbon tetrachloride in air at 94,500 mg/m^3 for 475 minutes. The dogs were

TABLE 7-2

Carbon Tetrachloride Distribution at Various Times in Anesthetized Dogs After Administration by Stomach Tube (mg/100 g of tissue)*

Tissue	6 hrs	23 hrs	24 hrs
Brain	--	17	9
Blood, portal	26	13	22
Blood, arterial	0	0	0
Bone marrow	--	66	--
Kidney	--	11	13
Liver	15	10	27
Lungs	--	trace	6
Muscle	--	trace	20
Pancreas	--	4.5	14
Spleen	--	5	--

*Source: Roobins, 1929

sacrificed at the end of the exposure. Tissue and blood samples were taken and analyzed for carbon tetrachloride. The concentration of carbon tetrachloride (per 100 g of tissue) was 66 mg/100 g in the brain, 50 mg/100 g in the heart, 36 mg/100 g in the liver and 34 mg/100 g in the blood; the concentration in the fat was not determined. The investigators stated that the accumulation of carbon tetrachloride in the brain was consistent with its high oil-water partition coefficient and resulted in its strong narcotic action.

McCollister et al. (1951) reported the tissue distribution of carbon tetrachloride in a Rhesus monkey exposed to 290 mg/m^3 of [^{14}C]carbon tetrachloride for 300 minutes. The tissue distribution, as calculated from the ^{14}C radioactivity, is shown in Table 7-3. The concentration of carbon tetrachloride was greatest in the fat, followed by the liver and bone marrow.

Fowler (1969) studied the distribution of carbon tetrachloride in the tissues of rabbits given the chemical by stomach tube. Five rabbits were given carbon tetrachloride (1 mℓ/kg bw) as a 20% (v/v) solution in olive oil. Analysis of the carbon tetrachloride by GC showed $\leq$.125 mg/kg hexachloroethane. The rabbits were sacrificed 6, 24 and 48 hours after treatment and the tissues analyzed for carbon tetrachloride by GC/ECD. Six hours after carbon tetrachloride was administered, the tissue concentrations (per kg of tissue) were 787 $\pm$ 289 mg/kg (standard error) in fat, 96 $\pm$ 11 mg/kg in liver, 21 $\pm$ 12 mg/kg in muscle and 20 $\pm$ 13 mg/kg in kidney. By 48 hours, these concentrations had dropped to 45 $\pm$ 12 mg/kg in fat, 4 $\pm$ 0.1 mg/kg in liver and 0.5 $\pm$ 0.3 mg/kg in kidney and muscles.

It is difficult to compare these four distribution studies, because species, sacrifice times, doses and routes of exposure varied. Moreover, not all important tissues were sampled [e.g., von Oettingen, et al. (1950) did

TABLE 7-3

Tissue Distribution of [^{14}C] Carbon Tetrachloride Inhaled by a Rhesus Monkey*

Tissue	Carbon Tetrachloride (mg/100 g of tissue)
Fat	2.46
Liver	0.94
Bone marrow	0.93
Blood	0.31
Brain	0.30
Kidney	0.23
Heart	0.14
Spleen	0.10
Muscle	0.06
Lung	0.04
Bone	0.04

*Source: McCollister et al., 1951

not sample the fat]; and the number of animals in two cases was small [e.g., Roobins (1929) reported results on three dogs; McCollister et al. (1951) reported results on one monkey]. Nonetheless, it appears that the concentration of carbon tetrachloride will be highest in fat, liver, bone marrow, blood and perhaps kidney or brain after administration by either oral or inhalation routes. Certainly more work could be done in this area.

7.3. METABOLISM

Chloroform was one of the first carbon tetrachloride metabolites to be described (Butler, 1961). Eight dogs were exposed to carbon tetrachloride by tracheal cannula at the rate of 8000 mg/hr into inhaled air for 3 hours. At the cessation of exposure, the exhaled air from the dogs was collected and analyzed by both gas chromatography and the Fujiwara reaction, a colorimetric procedure for the identification of chloroform. Chloroform was detected in the exhaled breath by both of these methods. The total amount of chloroform exhaled in 2 hours by each dog was estimated at 0.1 to 0.5 mg by GC analysis. Tissue homogenates were also shown to metabolize carbon tetrachloride to chloroform.

Evidence of metabolism to a free radical was suggested by studies showing hexachloroethane to be a carbon tetrachloride metabolite (Bini et al., 1975). Five Wistar rats were administered 160 to 800 mg carbon tetrachloride dissolved in liquid paraffin by gavage following a 24-hour fast. The animals were sacrificed 15 minutes to 8 hours after treatment. A graph displaying carbon tetrachloride concentrations in rat liver versus time showed the chemical at ≈0.9 mg/kg of tissue after 15 minutes and at maximal concentration (1.7 mg/kg) after 120 minutes. GC analysis showed that chloroform was maximal at 0.037 mg/kg after 15 minutes; after 4 hours it had declined to 0.007 mg/kg. Hexachloroethane was also present after 4 hours at

0.005 mg/kg. The authors explained the formation of both chloroform and hexachloroethane as carbon tetrachloride metabolites by proposing that the trichloromethyl free radical was the primary metabolite of carbon tetrachloride.

^{14}C-labeled carbon dioxide was detected in the exhaled air of Rhesus monkeys after a 344-minute exposure to [^{14}C]carbon tetrachloride at 290 mg/m^3 by inhalation (McCollister et al., 1951). The amount of [^{14}C]carbon dioxide exhaled during the 7-day period following exposure was reported to be 10 to 20% of the total radioactivity expired. The authors fitted these data to a straight line, integrated the resulting equation from 18 to 1800 hours (75 days) and estimated that 4.4 mg or 11% of the total amount of radioactivity eliminated was excreted as carbon dioxide.

Shah et al. (1979) studied the metabolism of [^{14}C]carbon tetrachloride by rat liver in vitro. Samples of liver homogenate equivalent to 0.167 g of tissue were incubated for 30 minutes at 37.5°C with 10 µmole of [^{14}C]-labeled carbon tetrachloride alone, and with either NADH or NADPH or both. [^{14}C]carbon dioxide was detected by scintillation counting. The results are shown in Table 7-4. The addition of NADPH or NADH, separately or as a mixture, appeared to result in substantial conversion of carbon tetrachloride to carbon dioxide.

Shah et al. (1979) tested for the possible formation of carbonyl chloride in hepatic carbon tetrachloride metabolism by adding L-cysteine to the in vitro rat liver system described above. Carbonyl chloride and L-cysteine are known to react chemically to form a condensation product, 2-oxothiazolidine-4-carboxylic acid. The presence of the condensation product was confirmed by thin-layer chromatography (TLC) and mass spectrometry (MS). The authors inferred from the presence of 2-oxothiazolidine-4-carboxylic

TABLE 7-4

Conversion of [^{14}C]Carbon Tetrachloride to [^{14}C]Carbon Dioxide by Rat Liver Homogenate*

Nucleotide Added	[^{14}C]CO_2 (nmole/g liver, mean ± standard deviation)
None	27 ± 5
NADH	373 ± 17
NADPH	464 ± 33
NADH + NADPH	472 ± 21

*Source: Snah et al., 1979

acid that carbonyl chloride was formed in the metabolism of carbon tetrachloride by rat liver microsomes. The authors postulated a mechanism of biotransformation for carbon tetrachloride which involved a sequential oxidation of carbon tetrachloride while bound to a heme (Figure 7-1). Release of bound intermediates then gave rise to different metabolites at the site of release.

Fowler (1969) detected hexachloroethane and chloroform in the tissues of rabbits administered carbon tetrachloride orally. As previously described, a total of 15 rabbits were given carbon tetrachloride at 1 mℓ/kg bw and sacrificed in groups of five at 6, 24 and 48 hours after exposure. Samples of fat, liver, kidney and muscle tissue were analyzed for chloroform and hexachloroethane by gas chromatography. The results of the analysis are shown in Table 7-5. The fat contained the highest amounts of hexachloroethane at each sampling time, but the highest concentrations of chloroform appeared in the liver.

For a complete discussion on metabolic pathways hypothesized to be mechanisms of toxicity, see Chapter 8, Section 8.3.

7.4. ELIMINATION

McCollister et al. (1951) studied the elimination of ^{14}C-labeled carbon tetrachloride from Rhesus monkeys exposed by inhalation at 290 mg/m^3 for 344 minutes. The total ^{14}C radioactivity in the blood decreased 12% during the first 10 minutes after exposure. Graphs of data from the analysis of blood samples obtained periodically for 10 to 12 days following exposure showed that the level of carbon tetrachloride in the blood decreased exponentially with time. At 10 days, the level of carbon tetrachloride in blood was ≈0.009 mg/100 g. The authors estimated that 21% of the total amount of carbon tetrachloride absorbed was eliminated in expired air during

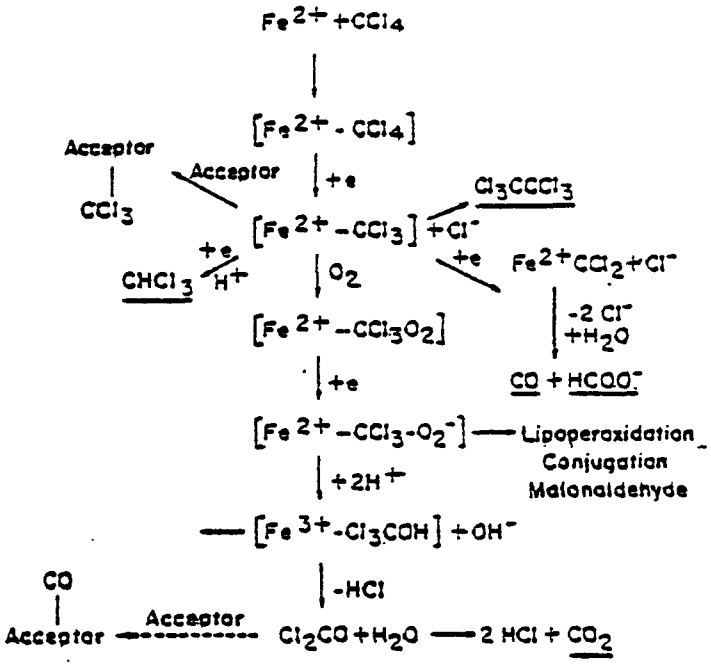


FIGURE 7-1

Pathways of Carbon Tetrachloride Metabolism. Products identified as carbon tetrachloride metabolites are underlined. The electrons utilized in the reactions are assumed to come from NADH or NADPH via the flavoprotin cytochrome reductases. Fe^{2+} and Fe^{3+} denote the respective ferro- and ferricytochromes.

Source: Redrawn from Shan et al., 1979

TABLE 7-5

Chloroform and Hexachloroethane in Tissues of Rabbits Given Carbon Tetrachloride Orally*

Sample Time	Tissue	$CHCl_3$ (µg/g tissue)	Cl_3CCCl_3 (µg/g tissue)
6 hours	Fat	4.7 ± 0.5	4.1 ± 1.2
	Liver	4.9 ± 1.5	1.6 ± 0.5
	Kidney	1.4 ± 0.6	0.7 ± 0.2
	Muscle	0.1 ± 0.1	0.3 ± 0.2
24 hours	Fat	1.0 ± 0.2	16.5 ± 1.6
	Liver	1.0 ± 0.4	4.2 ± 1.8
	Kidney	0.4 ± 0.2	2.2 ± 1.1
	Muscle	0.1 ± 0.1	0.5 ± 0.2
48 hours	Fat	0.4 ± 0.1	6.8 ± 2.4
	Liver	0.8 ± 0.2	1.0 ± 0.3
	Kidney	0.2 ± 0	Trace
	Muscle	0.1 ± 0.1	Trace

*Source: Fowler, 1969

the first 18 days. By extrapolation of these data, the authors concluded that after 1800 hours (75 days), approximately 51% of the carbon tetrachloride initially absorbed would be eliminated in exhaled breath either as carbon tetrachloride or carbon dioxide. Analysis of urine and feces showed measurable amounts of radioactivity after 15 and 12 days, respectively. The authors interpreted these findings as indicating that significant quantities of carbon tetrachloride and/or metabolites may be excreted by these routes.

7.5. SUMMARY

Carbon tetrachloride is readily absorbed from the lungs and the gastrointestinal tract, as expected from its partition coefficients. Although few quantitative data are available on the amount of carbon tetrachloride absorbed through the lungs, the chemical and its metabolites have been reported in blood, many tissues, exhaled air, urine and feces after administration by this route. Carbon tetrachloride is also absorbed through the skin.

The best available data concerning CCl_4 absorption come from two studies and one report:

- 30.4% via inhalation in monkeys (McCollister et al., 1951)
- 57 to 65% via inhalation in humans (Lehmann and Schmidt-Kehl, 1936)
- 30% via inhalation and 50% via ingestion (Stokinger and Woodward, 1958).

The Stokinger and Woodward report has been criticized for not including substantiating information or alternatively, citing the specific literature. There are some considerations associated with extrapolating from animal data to humans in that animals may alter their breathing patterns during an experiment or may not be good models insofar as their metabolism is concerned. This is not to say that the animal data or Stokinger and Woodward

report should be overlooked. Therefore, it is recommended that a 40% absorption coefficient be used when the route of exposure is via inhalation and that a 100% absorption coefficient be used when the route of exposure is via ingestion. The former being a compromise based upon the available information and the latter being the conservative estimate due to little information.

In the studies reviewed, carbon tetrachloride appears to be distributed to all major organs following absorption. The highest concentrations have been found in the fat, liver, bone marrow, blood, brain and kidney.

Carbon tetrachloride metabolism has been reported to occur primarily in the liver. Carbon tetrachloride has been postulated to be metabolized to a trichloromethyl radical bound to an iron atom in the cytochrome heme moiety. This trichloromethyl radical is thought to be either further metabolized or released as a free radical. It is suggested that the trichloromethyl free radical can undergo a variety of reactions, including macromolecular binding, hydrogen abstraction to form chloroform, and dimerization to form hexachloroethane.

Carbon tetrachloride and its metabolites have been reported in many studies to be excreted primarily in exhaled air, but also in the urine and feces. However, pharmacokinetic data on these processes are apparently lacking.

8. TOXICOLOGY: ACUTE, SUBCHRONIC AND CHRONIC

Hepatotoxicity is the major effect reported to be produced by acute exposure of animals to carbon tetrachloride. Hepatic necrosis and fatty liver degeneration have been documented after both inhalation exposure and ingestion. Hepatotoxicity has sometimes been accompanied by kidney and lung effects. In addition, prenatal toxic effects have been demonstrated after inhalation of the chemical by pregnant rats. This chapter discusses the effects from acute exposure (single dose, one day or several days defined in rodents), subchronic exposure (2 weeks to somewhat more than 90 days defined in rodents) and chronic exposure ($\geq$6 months defined in rodents) to carbon tetrachloride. Emphasis is placed on studies in which dose/response relationships have been developed. Toxic responses occurring prior to carcinogenicity are reported in Chapter 11.

8.1. EXPERIMENTAL ANIMALS

8.1.1. Acute. The toxicity from acute exposure to carbon tetrachloride has been documented extensively. Because defining the dose range that produces minimal health effects is an objective of this report, this section will concentrate on those studies that (1) describe nonlethal effects and (2) provide data on a range of doses from which dose-response relationships can be determined. For this reason a number of studies referring to LD_{50}s will not be discussed. Table 8-1 summarizes some of the lethal dose data reported for carbon tetrachloride in various species.

Administration of carbon tetrachloride results in a number of acute systemic effects. Its hepatic effects are the most pronounced: carbon tetrachloride causes necrosis and fatty liver degeneration. The hepatic effects caused by carbon tetrachloride have been well documented in many scientific studies. However, toxicity to other organs has also been described. The following subsections will focus on such organ toxicity.

TABLE 8-1

Toxic Doses and Effects of Carbon Tetrachloride in Animals[a]

Animal	Route of Administration	Effect[b]	Dose
Rat	Oral	LD_{50}	2,800 mg/kg
Mouse		LD_{50}	12,800 mg/kg
Dog		LD_{Lo}	1,000 mg/kg
Rabbit		LD_{50}	6,380 mg/kg
Rat	Intraperitoneal	LD_{50}	1,500 mg/kg
Mouse		LD_{50}	4,675 mg/kg
Dog		LD_{Lo}	1,500 mg/kg
Rabbit		LD_{Lo}	478 mg/kg
Rat	Inhalation	LC_{50}	624.80 mg/m^3
Mouse		LC_{50}	1,487.97 mg/m^3
Cat		LC_{Lo}	5,952.83 mg/m^3
Guinea pig		LC_{Lo}	3,124.02 mg/m^3
Cat	Subcutaneous	LD_{Lo}	300 mg/kg
Rabbit		LD_{Lo}	3,000 mg/kg

[a]Source: NIOSH, 1978

[b]LD_{50}, dose lethal for 50% of animals
LC_{50}, concentration lethal for 50% of animals
LD_{Lo}, lowest lethal dose
LC_{Lo}, lowest lethal concentration

8.1.1.1. **LIVER EFFECTS** -- Functional changes in mouse liver as a result of carbon tetrachloride exposure were measured by increases in activity of the enzyme serum glutamic-pyruvic transaminase (SGPT) and in bromsulfophthalein (BSP) retention (Klaassen and Plaa, 1966). Male Swiss-Webster mice were administered various amounts of analytical grade carbon tetrachloride i.p. in corn oil in a final volume of 10 mL/kg bodyweight (bw). Mice treated only with corn oil were used to establish the normal range of values for BSP retention and SGPT activity, which were determined 24 hours after treatment. The authors reported the median effective doses of carbon tetrachloride as 15.9 mg/kg bw for elevation of SGPT activity and 94 mg/kg bw for BSP retention. The authors did not report the range of carbon tetrachloride doses used or the number of animals used at each dose.

In an additional study, Klaassen and Plaa (1967) further defined a dose-response relationship for carbon tetrachloride exposure and elevated SGPT levels in mice. They used the "up and down" method in which one dose of the compound was given intraperitoneally; the animal's SGPT activity 24 hours after the dose was noted. If the enzyme activity was elevated, the dose was decreased 40% and the experiment repeated in another animal. If no effect was noted, the dose was increased 40% and the experiment repeated in another animal. This series was repeated three times after one positive and one negative response had been obtained. The results for mice are shown in Table 8-2. The authors concluded that 13 mg/kg bw was the median effective dose (ED_{50}) of carbon tetrachloride in mice as measured by elevated SGPT values.

Sein and Chu (1979) studied the effect of carbon tetrachloride on the level of the liver enzyme glucose-6-phosphatase in mice. Groups of six male LAC strain mice were treated i.p. with 795, 1590 or 3180 mg/kg bw carbon

TABLE 8-2

SGPT Values of Mice Administered Carbon Tetrachloride Intraperitoneally in "Up and Down" Experiment[a]

Animal	Dose (mg/kg bw)	Response[b]
1	17.5	E
2	13	N
3	17.5	E
4	13	E
5	8	N
ED_{50}	13	

[a]Source: Klaassen and Plaa, 1967

[b]E = Elevated SGPT after 24 hours
N = Normal SGPT after 24 hours

ED_{50} = Median effective dose

tetrachloride (purity unspecified) in paraffin oil. The animals were sacrificed 24 hours after treatment. Control animals (number unspecified) were given paraffin oil and sacrificed on the same schedule. The livers were removed and analyzed for glucose-6-phosphatase. The results of the analysis showed that after treatment with carbon tetrachloride at 795 or 1590 mg/kg, the enzyme level fell to 40% of the control value. At a dose of 3180 mg/kg, the enzyme level had decreased to 20% of the control value.

A series of experiments to determine the effects of single carbon tetrachloride exposures on rats were performed by Murphy and Malley (1969). Adult male Holtzman rats (250 to 350 g) were orally administered various doses of undiluted carbon tetrachloride by gavage. Control animals were administered equal volumes of water. At 2 to 20 hours after treatment, animals were sacrificed and liver enzyme activities and liver weights were measured. The results are shown in Table 8-3. The animals receiving carbon tetrachloride at 1600 mg/kg bw were sacrificed 20 hours after treatment and the livers examined histopathologically. The examination showed extensive fatty infiltration, inflammation and some centrilobular necrosis. The liver-to-body weight ratios were also increased.

Murphy and Malley (1969) also determined the effect of single exposures to carbon tetrachloride on the activities of the corticosterone-inducible liver enzymes tryptophan pyrrolase and tyrosine-ketoglutarate transaminase. Groups of rats (4 to 6 in each group, 8 untreated controls) were treated with carbon tetrachloride (0, 400, 800 and 1600 mg/kg bw by gavage) and sacrificed 5 hours after treatment. Data graphs showed that the enzyme levels were increased roughly in proportion to the dose.

Similar studies on the effect of carbon tetrachloride administration on serum activity of liver enzymes in rats were performed by Drotman and

TABLE 8-3

Effects of Oral Carbon Tetrachloride on Liver Weight and Liver and Plasma Enzyme Activities in Male Rats[a]

Dose (mg/kg bw)	Time (hr)	Number of Animals	Plasma AKT[b]	Liver[c]			
				AKT[b]	TKT[b]	AP[b]	Weight (g/100g bw)
0	-	7	2.6 ± 0.3[c]	2360 ± 182	93 ± 11	14.9 ± 1.0	2.75 ± 0.06
3200	2	4	2.1 ± 0.2	-	170 ± 12	14.5 ± 1.0	2.94 ± 0.04
3200	5	5	13.2 ± 3.4	1813 ± 331	330 ± 36	14.2 ± 1.3	3.26 ± 0.10
3200	20	5	35.2 ± 4.2	1174 ± 559	361 ± 42	34.6 ± 5.8	4.36 ± 0.06
2400	20	4	35.2 ± 3.4	1585 ± 148	305 ± 21	37.1 ± 1.6	3.95 ± 0.07
1600	20	5	18.1 ± 4.3	1596 ± 194	294 ± 61	33.3 ± 3.6	3.90 ± 0.05
800	20	4	9.3 ± 2.3	2120 ± 182	138 ± 5	29.1 ± 6.5	3.47 ± 0.07

[a]Source: Adapted from Murphy and Malley, 1969

[b]AKT = alanine-α-ketoglutarate transaminase
TKT = tyrosine-α-ketoglutarate transaminase
AP = alkaline phosphatase

[c]Means ± SE in micromoles of product formed per gram of fresh liver or milliliter of plasma per hour

Lawhorn (1978). Groups of four male Cox rats were administered carbon tetrachloride i.p. at 60, 120, 240 or 480 mg/kg bw in a total volume of 1 mℓ in corn oil and exsanguinated at specified time intervals. Serum activities were determined for the enzymes sorbitol dehydrogenase (SSDH), ornithine carbamyl transferase (SOCT), aspartate aminotransferase (SAST) and isocitric dehydrogenase (SICDH). Liver specimens were taken from each animal and scored for histopathological changes. The results of the enzyme analyses and histopathology are tabulated in Table 8-4 by dose and hours after dose. The SOCT activities showed the best correlation with liver histopathology in time of appearance as well as extent of damage. The authors concluded that SOCT levels are a sensitive indicator of liver damage.

Effects of acute exposure to low levels of carbon tetrachloride were also reported by Korsrud et al. (1972). Male Wistar rats (260 to 400 g; 8 to 10 animals per treatment group) were administered single oral doses of carbon tetrachloride (0 to 4000 mg/kg bw) in corn oil (5 mℓ/kg bw). The rats were fasted for 6 hours before treatment and for 18 hours afterward, and then sacrificed. Assays included liver weight and fat content, serum urea and arginine levels, and levels of nine serum enzymes produced mainly in the liver. At 20 mg/kg bw there was histopathologic evidence of toxic effects on the liver. These changes included a loss of basophilic stippling, a few swollen cells and minimal cytoplasmic vacuolation. At 40 mg/kg bw, liver fat, liver weight, serum urea and levels of 5 of the 9 liver enzymes were increased while serum arginine decreased. At higher doses the remaining four enzyme levels were also elevated.

In addition to the study on hepatic effects of carbon tetrachloride in mice described earlier in this section, Klaassen and Plaa (1967) also investigated the hepatic effects of carbon tetrachloride exposure on dogs. Male

TABLE 8-4

Effects of Carbon Tetrachloride on Liver Histopathology and Serum Enzyme Levels[a]

Dose (mg/kg)	Hours After Dose	Histology[b]	Serum Enzyme Concentrations Relative to Pretreatment Levels			
			SOCT	SSDH	SAST	SICDH
60	0	0	1.0	1.0	1.0	1.0
	6	2	9.6*	2.5	2.0	1.4
	12	1	8.2*	4.4*	2.5*	1.1
	24	1	5.7*	1.7	2.0	1.3
	36	0	1.0	1.0	1.0	1.0
120	0	0	1.0	1.0	1.0	1.0
	24	3	14.0*	7.2*	2.1*	1.1
	48	2	7.4*	1.0	1.0	1.0
	96	1	1.8	1.7	1.3	1.3
	168	0	1.0	1.0	1.0	1.0
240	0	0	1.0	1.0	1.0	1.0
	24	3	31.0*	17.4*	5.8*	7.2*
	48	4	180.0*	43.4*	17.0*	7.4
	96	1	6.6*	4.7*	3.6*	2.0
	168	0	1.1	1.0	1.9	2.0
480	0	0	1.0	1.0	1.0	1.0
	24	3	28.4*	90.0*	6.1*	5.4*
	48	4	465.5*	163.5*	18.4*	50.4*
	96	1	1.0	8.4*	1.8*	2.0
	168	0	1.0	1.4	1.0	2.1

[a]Source: Adapted from Drotman and Lawhorn, 1978

[b]0 = No observable changes.
1 = Minimal changes. Large central vein, swelling of hepatocyte, etc.
2 = Mild degenerative change. Loss of cord arrangement.
3 = Moderate degenerative change. Pale cytoplasm, spindle cell.
4 = Marked degenerative change. Centrilobular fatty degeneration.

*Significantly different from zero time as determined by one-way analysis of variance of the log-transformed data ($p \leq 0.01$).

and female mongrel dogs were treated intraperitoneally with carbon tetrachloride at 22 to 38 mg/kg bw in an "up and down" experimental design. Blood samples were taken for measurement of SGPT 24 hours after administration of carbon tetrachloride. Control dogs had serum SGPT activity of 36 $\pm$ 7 units. Therefore, 36 + 2 standard deviations or 50 units was chosen as the upper limit of the normal value. The results of the analysis are shown in Table 8-5. The SGPT returned to normal in 17 to 18 days. Animals were then sacrificed and the livers were examined histopathologically. They showed moderate vacuolation of the centrilobular and midzonal hepatocytes as well as traces of brown material in the cytoplasm of centrilobular Kupffer cells.

Gardner et al. (1924) also studied the acute toxic effects from ingestion of CCl_4 in dogs. Effects ranged from no apparent effect at 0.01 mℓ/kg (15.89 mg/kg) to centrilobular necrosis at 0.05 mℓ/kg (79.45 mg/kg). Rabbits similarly treated experienced liver necrosis at 0.1 mℓ/kg (158.9 mg/kg).

Kronevi et al. (1979) administered CCl_4 epicutaneously to guinea pigs. Dermal effects were seen (see Section 8.1.1.4.) along with effects on the liver. Liver morphology was characterized by hydropic and necrotic changes. Altered liver morphology was seen after 16 hours when hepatocytes in the central two-thirds of each lobule showed marked hydropic changes which were characterized by large, clear cytoplasmic spaces. It was noted that there was also a tendency to necrotic lesions characterized by homogeneous, slightly eosinophilic and slightly PAS-positive structures within the cytoplasm. Glycogen was absent and the nuclei showed a tendency to degeneration (Kronevi et al., 1979).

TABLE 8-5

SGPT Activity in Dogs 24 Hours After Intraperitoneal Administration of Carbon Tetrachloride in "Up and Down" Experiment[a]

Animal	Dose (mg/kg)	Response[b]
1	22.2	N
2	30.2	E
3	22.2	N
4	30.2	N
5	38	E
ED_{50}	32	

[a]Source: Adapted from Klaassen and Plaa, 1967

[b]N = normal SGPT after 24 hours
E = elevated SGPT after 24 hours

ED_{50} = Median effective dose

In studying the renal effects of CCl_4 in cats (see Section 8.1.1.2.), Wong and DiStefano (1966) noted a delayed liver reaction. Liver weights were significantly increased 24 hours following exposure by inhalation.

8.1.1.2. KIDNEY EFFECTS -- In experiments conducted by Plaa and Larson (1965) using CCl_4, high doses failed to induce renal failure as measured by phenolsulfonphthalein (PSP) excretion in mice although pathological kidney alterations were present. Male Swiss mice (18 to 30 g) were given i.p. injections of carbon tetrachloride (1600 to 6400 mg/kg bw) dissolved in corn oil at a final amount of 10 mg/kg bw. The animals were then hydrated with tap water (50 mg/kg bw) by gavage and placed on a urinary collection unit for 2 hours. Even carbon tetrachloride doses lethal in some animals ($\geq$6400 mg/kg bw) failed to cause renal dysfunction, measured as excretion of PSP, urinary protein and glucose, in the majority of survivors. At a high nonlethal dose (3260 mg/kg bw), minimal renal dysfunction was observed after 96 hours. Histologic examination of kidney sections from five mice that had been administered this dose showed that one of the mice showed necrosis of proximal convoluted tubules, and 4 of 5 mice showed swelling of the tubules.

Carbon tetrachloride did, however, decrease activity of glucose-6-phosphatase in the kidney (Sein and Chu, 1979). Male mice (40 to 50 days old, weigning 24 to 28 g) were injected i.p. with carbon tetracnloride at 795, 1590 or 3180 mg/kg bw in paraffin oil. Twenty-four hours after injection of 795 or 1590 mg/kg bw, the kidney glucose-6-phosphatase activity decreased to 77 or 65% of the control value, respectively. Increasing the dose to 3180 mg/kg bw had no further effect on the kidney enzyme level.

These results were in contrast to the liver glucose-6-phosphatase level discussed earlier, which decreased to 40% of the control value at the two

lower doses and decreased further to 20% of the control value at the 3180 mg/kg bw dose. The authors attributed these differences to the limited metabolic capacity of the kidneys.

Klaassen and Plaa (1967) studied the effect of carbon tetrachloride exposure on kidney function in dogs. PSP excretion of <39% of control value was considered indicative of renal dysfunction. An unspecified number of male and female mongrel dogs were treated i.p. with carbon tetrachloride at 22 to 38 mg/kg bw, and the 24-hour excretion rate for PSP was determined. Control dogs were used to determine a normal range for PSP excretion. None of the dogs treated with carbon tetrachloride exhibited decreased PSP excretion. However, on histopathic examination of the kidneys from the treated dogs, the Bowman's capsules appeared dilated with some contraction of glomerular tufts and calcification of a small number of tubules in the medulla.

Striker et al. (1968) examined the structural and functional changes in the rat kidney during CCl_4 intoxication. Exposure was 2.5 mℓ/kg bw in an equal volume of mineral oil by gastric intubation. A progressive increase in the size and paleness of the kidney was apparent during the first 2 days after exposure, with maximum effects seen at 48 hours. Alterations were limited to the proximal tubule and appeared to involve primarily the middle and lower segments. The alterations seen were sequential and reversible. The earliest morphological change was in the mitochondria, followed by cellular swelling manifested by loss of basilar interdigitations and swollen microvilli. Occurring later was the appearance of large aggregates of smooth-surfaced membranous profiles. By 5 days after exposure, all alterations were reversed (Striker et al., 1968).

Also apparent were differences in serum parameters reflecting kidney function. Serum creatinine and blood urea nitrogen peaked at 12 hours and 24 hours, respectively. Total serum bilirubin was elevated 12 to 48 hours. By 5 days all values returned to normal (Striker et al., 1968).

Wong and DiStefano (1966) examined lipid accumulation and histologic cnanges in tne kidney of tne cat following CCl_4 inhalation. Kidney weight:body weight ratios increased significantly following 60- and 240-minute exposures. The renal cortical lipid content increased significantly within 15 minutes and an accumulation of fat droplets was seen histologically in 30 to 60 minutes. One hour after the termination of CCl_4 inhalation, the kidneys were enlarged and the weight continued to increase past the 24-hour observation period (Wong and DiStefano, 1966).

8.1.1.3. LUNG EFFECTS -- Boyd et al. (1980) investigated the effect of ingestion and inhalation of carbon tetrachloride on pulmonary Clara cells in Swiss mice. For the ingestion study, the mice were treated with carbon tetrachloride (4000 mg/kg bw) in a 50% sesame oil solution and sacrificed 16 hours after treatment. The lungs were removed and examined by electron microscopy. The examination showed the Clara cells to have massive dilation of vesicles of smooth endoplasmic reticulum, increased mitochondrial staining density, ribosomal disaggregation, nuclear condensation and occasional cellular necrosis. Additional experiments with oral carbon tetrachloride doses of <1600 mg/kg bw did not produce any pulmonary lesions visible by light microscopy. Doses of 2400 to 4800 mg/kg bw produced Clara cell lesions similar on electron microscopic examination to those previously described. The extent of damage was proportional to the dose administered. Boyd et al. (1980) also studied the time course of the Clara cell damage

caused by ingestion of carbon tetrachloride (4000 mg/kg bw). Pulmonary tissue was evaluated by light microscopy at 12, 24, 36, 48, 96 and 168 hours. The lesions were present at 12 hours, maximal at 24 hours, and less intense at 36 hours. By 48 hours, the lesions were seen infrequently, and at 96 and 168 hours the pulmonary bronchioles appeared normal.

The pulmonary toxicity of inhaled carbon tetrachloride was also studied by Boyd et al. (1980). Swiss mice were exposed to carbon tetrachloride vapor at 71,800, 144,000, 287,000 or 574,000 mg/m^3 for 60, 60, 12 or 2 minutes, respectively. The animals were sacrificed 24 hours after exposure, and the lungs were examined. Marked Clara cell lesions similar to those seen after oral exposure were seen at all exposure levels; necrosis was reported to be more frequent after inhalation than after oral exposure, but no effort to quantify this finding was reported.

Gould and Smuckler (1971) detailed the structural alterations in rat lungs following carbon tetrachloride ingestion. Male Sprague-Dawley rats (200 to 250 g) were fasted 16 hours prior to administration of carbon tetrachloride (4000 mg/kg bw) by gavage. The animals exhibited piloerection and lassitude 3 to 4 hours after treatment. Necropsies were performed on all animals. Microscopic examination of the lungs of treated rats revealed perivascular edema and mononuclear infiltration in the first 4 hours after treatment. These areas were local but were estimated to involve 10% of the parenchyma. Areas of atelectasis and intraalveolar hemorrhage involving 15 to 20% of the parenchyma were observed 8 to 12 hours after treatment.

Electron micrographs of rat lungs after carbon tetrachloride ingestion (Gould and Smuckler, 1971) showed granular pneumocytes containing swollen inclusions with decreased osmiophilia and attenuated lamellae 1 hour after treatment. These changes were more severe 4 hours after treatment. By 4 to

8 hours after treatment, cytoplasmic edema, dislocation of dense ribosomal aggregates and mitochondrial disruption were apparent. Multivesicular bodies were "conspicuously decreased" within the granular pneumocytes. Necrosis was evident 12 to 24 hours after treatment. One hour after administration, endothelial cells displayed markedly increased pinocytotic vesicles. Severe disruption of endothelial cells was evident from 8 hours onward. Ultrastructural damage was seen in all components of the alveolar wall, and fibrin was observed within alveoli. The authors interpreted these findings as showing significant alterations in vascular permeability.

Lesions of the Clara cells in the lungs of male Sprague-Dawley rats orally treated with carbon tetrachloride were observed by Boyd et al. (1980). The carbon tetrachloride was administered by gavage at doses of 3816, 5088 and 7155 mg/kg as a 50% solution in sesame oil. Control animals received sesame oil only. Clara cell lesions occurred at the two highest doses. The authors stated that the lesions were less pronounced than those in mice exposed to comparable amounts of carbon tetrachloride.

Chen et al. (1977) examined the effects upon the lungs of rats given a single CCl_4 exposure by gastric intubation or by inhalation. Exposures were: 2.5 mℓ/kg bw by gastric intubation, and 30 minutes in air containing 4.38% CCl_4 (280,400 mg/m^3) by inhalation. Both the orally administered and innaled CCl_4 markedly modified the lung and liver cytochrome P-450 content, but there was a greater response in the pulmonary tissue. Inhalation resulted in a less significant depression of activity in both organs (Chen et al., 1977). Morphologic analyses of the lung revealed focal changes by 1 to 7 days in pulmonary architecture consisting of areas of alveolar collapse, septal thickening, transudation and modification of type II pneumonocytes.

8.1.1.4. **DERMAL EFFECTS** -- Kronevi et al. (1979) examined the effects on guinea pigs following epicutaneous administration of 1 mℓ CCl_4. Slight karyolysis was seen. After 15 minutes, marked spongiosis developed. Fifteen minutes and onward, karyopyknosis was evident. The authors saw progressing nuclear pyknosis and junctional separation between the basement membrane and the basal cells along with induced spongiosis appearing before the junctional separation attributed to the CCl_4 exposure.

8.1.1.5. **BIOCHEMICAL AND OTHER EFFECTS** -- Merkureva et al. (1979) studied the effects of continuous inhalation of CCl_4 on the rat enzyme system. Exposure of 300 mg/m^3 CCl_4 in air resulted in 55% reduction in the activity of N-acetyl-β-D-glucosaminidase 24 hours following initiation. The activity of hyaluronidase of blood serum was increased by 96% 3 days after exposure. The activities of β-glucosidase, acid phosphatase and β-galactosidase were also affected. Finally, a 39% inhibition of N-acetylneuraminic acid aldolase was seen.

Following injection of CCl_4, Wyrebowska and Jerzykowski (1980) saw a change in enzyme activity in rat serum. Mature male and young Wistar rats were given a single 2 mℓ/kg bw dose of CCl_4 i.p. dissolved in sterile vegetable oil (1:1). Following this dose, both the alkaline and acid forms of aminopropanol dehydrogenase appeared in the blood serum. Maximum activity occurred 24 hours after administration in the young rats and 12 hours after administration in the mature male rats.

Mikhail et al. (1978) injected male and female adult rats intraperitoneally with 0.5 mℓ of a 1:1 mixture of CCl_4 in mineral oil per 100 g bw (0.005 mℓ of the mixture per g bw). This resulted in an increase in serum iron, copper, zinc, calcium, potassium and sodium 24 hours after administration. There was no change observed in serum magnesium. The authors attrib-

ute the rise in some serum chemistries to the known hepatotoxic effect of CCl_4. They conclude that the disturbance in minerals mitabolism is one of the earliest lesions in CCl_4 poisoning (Miknail et al., 1978).

David et al. (1981) examined the effect of different exposure schemes upon biochemical and morphological changes in the rat liver. Groups of six male Wistar rats, 7 months old, were exposed to CCl_4 vapors with differing exposure schedules. Schedules were designed in such a way to give a constant product of concentration and time (CT = 1950 mg/m^3) for 4 successive days a week.

It was found that a higher dose given over a shorter period of time had a greater effect than a lower dose given over a longer period of time (1625 mg/m^3 for 72 minutes vs. 325 mg/m^3 for 6 hours). Also, continuous exposure (18 minutes) to 6500 mg/m^3 produced a greater effect than intermittent exposure (3 minutes, 6 times with 1 hour intervals) to 6500 mg/m^3. The authors conclude that sensitivity of the liver is more influenced by the concentration of CCl_4 in the inhaled air than in the total amount inhaled, the theory being that the former allows more CCl_4 into the blood entering the liver. As the authors explain, this information is important in putting time weighted averages (TWAs) in the workplace.

8.1.2. Subchronic. The toxicity following subchronic exposure to CCl_4 has not been as extensively described as the toxicity following acute exposure. Paquet and Kamphausen (1975) examined biochemical changes in rats following subchronic exposure to CCl_4. Administration of CCl_4 was by subcutaneous injection of 1 mℓ/kg bw in an equal amount of peanut oil at 7-day intervals for 8 weeks. The authors describe the changes in stages. In stage 1 there is a decrease in pseudocholinesterasis indicative of the stage of liver necrosis. In stage 2, the triglycerides reach a high pla-

teau, there is an increase in SGOT, an increase in BSP retention and a continued decrease in pseudocholinesterasis. The second stage is characterized by massive fatty infiltration of the liver and increased necrosis with liver fibrosis at the end. In stage 3, SGOT continues to increase along with hydroxyproline, triglycerides and BSP retention. This final stage is characterized by a reduced synthetical ability and atrophy of the liver.

Bizin et al. (1977) performed a comparative study of the effect on rats of continuous and intermittent exposure to CCl_4. Continuous inhalation of 500 mg CCl_4/m^3 for 10 days induced toxicity symptoms 4- to 5-fold as rapidly as did inhalation for 6 hours daily for 40 days (Bizin et al., 1977).

Additionally, Alumot et al. (1976) reported the effects upon groups of six weanling rats 4 weeks old fed a diet containing carbon tetrachloride at 150, 275 or 520 mg/kg of feed for 5 weeks (females) or 6 weeks (males). The fumigated feed was stored in airtight containers; carbon tetrachloride loss during the storage period of 7 to 10 days was determined to be 5%. The animals were allowed access to the feed only at set time intervals to minimize loss of carbon tetrachloride by volatilization. The authors calculated that the amount of carbon tetrachloride remaining in the consumed feed was 60 to 70% of the amount initially present; the total decrease reflected amounts lost during storage and after removal from storage to feeding troughs. From these data and the weights of the animals, the authors calculated that 275 mg/kg of feed represented a daily dose of 40 mg/kg bw. By assuming that all parameters were the same and that the delivered dose was proportional to the concentration in feed, diets of 150 and 520 mg/kg of feed can be calculated to represent daily doses of 22 and 76 mg/kg bw, respectively (U.S. EPA, 1981). At the end of the experiment the animals were weighed and killed. Of the three doses, only the highest, 76 mg/kg bw (520 mg/kg of feed),

caused significantly depressed weight gain in males. Weight gain in females appeared to be unaffected by all doses. Total lipid and triglyceride levels in the liver were significantly higher in animals fed carbon tetrachloride at 40 and 76 mg/kg bw than in controls or animals fed 22 mg/kg bw. Levels of liver phospholipids (measured in females) were not affected at any dose. Of the three doses used in this experiment, the lowest (22 mg/kg bw) failed to produce effects on the measured parameters.

Prendergast et al. (1967) repeatedly (8 hours/day, 5 days/week) exposed 15 guinea pigs to carbon tetrachloride (purity unspecified) at 515 mg/m^3 over a period of 6 weeks and observed hepatic changes. Three guinea pigs died on days 20, 22 and 30. All the animals showed a body weight loss. The surviving animals were sacrificed at 6 weeks and the livers examined histopathologically. The examination revealed fatty infiltration, fibrosis, bile duct proliferation, hepatic cell degeneration and regeneration, focal inflammatory cell infiltration, alteration of lobular structure and early portal cirrhosis. The lipid content of the guinea pig liver was reported to be 35.4 $\pm$ 10.7%, much higher than the control value of 11.0 $\pm$ 3.6%.

In addition, Prendergast et al. (1967) also continuously exposed guinea pigs to carbon tetrachloride vapor at 61 mg/m^3 for 90 days. Three of the 15 guinea pigs died on days 47, 63 and 74. All the exposed animals showed a depressed weight gain. A "high incidence" of enlarged and discolored livers was reported on gross pathological examination. Histopathologic examination of the livers revealed fatty changes, fibroblastic proliferation, collagen deposition, hepatic cell degeneration and regeneration, and structure alteration of the liver lobule. Enzymatic studies showed that only the succinic dehydrogenase (SDH) activity was moderately reduced as compared to that in controls.

Prendergast et al. (1967) also exposed guinea pigs, number unspecified, to carbon tetrachloride vapor continuously at 6.1 mg/m^3 for 90 days. The authors reported that "no visible signs of toxicity" were seen during this study. Lipid content of the liver and serum urea nitrogen were within normal range. The authors concluded that no pathologic changes could be attributed to carbon tetrachloride exposure.

In addition to their studies on guinea pigs, Prendergast et al. (1967) studied the effects of both repeated and continuous exposure to carbon tetrachloride on three squirrel monkeys, three New Zealand rabbits, and two beagle dogs for each exposure regime. The experimental designs were the same as those described for the guinea pigs. All the animals showed a weight loss during repeated exposure to 515 mg/m^3. Fatty changes were noted in the liver of all species; they were most severe in rabbits, followed by dogs and monkeys. In the continuous exposure to 61 mg/m^3 for 90 days, all species exhibited a depressed weight gain, as did guinea pigs. Liver changes were also noted, but enzyme activities (as measured by NADH, NADPH, SDH, LDH and G6PD) were within the normal range. At a continuous exposure of 6.1 mg/m^3, no toxic signs were noted.

Finally, the same investigators (Prendergast et al., 1967) studied the effects of repeated (515 mg/m^3) and continuous (61 mg/m^3 or 6.1 mg/m^3) exposure of rats following the same methodology described above. With repeated exposure, there was a high percentage of mottled livers. Histopathologic examination revealed morphologic changes in lungs and livers but no changes in the heart, spleen or kidney. Fatty changes also developed in the liver. Following continuous exposure to 61 mg/m^3, depressed growth curves resulted as compared to control animals. Examination upon autopsy revealed a high incidence of enlarged and/or discolored livers. Histopathologic

liver changes included fatty infiltration, fibroblastic proliferation, collagen deposition, hepatic cell degeneration and regeneration, and alteration in the structure of the liver lobule. There were no adverse effects following continuous exposure to 6.1 mg/m^3.

The studies done by Prendergast et al. (1967) have been criticized due to small sample size (only rats and guinea pigs had numbers >10), inconsistent reporting (over 10 different descriptions of liver damage are mentioned) and vague information such that only the general conclusion that liver damage follows CCl_4 inhalation can be made (EnviroControl, 1981).

8.1.3. Chronic. Smyth et al. (1936) studied the toxicity of carbon tetrachloride on rats after chronic inhalation exposure. Groups of 24 Wistar rats were exposed to carbon tetrachloride concentrations of 315, 630, 1260 or 2520 mg/m^3 for 8 hours/day, 5 days/week for 10.5 months. The CCl_4 was found to contain <0.003% carbon disulfide. Control rats were used, but the number was unspecified. Growth retardation was observed with the 2520 mg/m^3 dose. At the 630 and 1260 mg/m^3 dose, growth was the same as in controls, and at the 315 mg/m^3 dose the growth was stimulated. Cirrhosis developed in the 630, 1260 and 2520 mg/m^3 groups after 173, 115 and 54 exposures, respectively, but not in the 315 mg/m^3 group. When exposure was stopped fatty liver degeneration resolved within 50 days. Surface alterations (hobnail liver) did not resolve until 156 days after cessation of exposure. Unspecified renal damage was observed after 52 exposures to the 315 mg/m^3 concentration and after 18 to 20 exposures at the higher concentrations, but was termed "not extreme."

In this same study (Smyth et al., 1936), groups of 24 guinea pigs were exposed to carbon tetrachloride vapor at 315, 635, 1260 or 2520 mg/m^3. The frequency of exposure was 8 hours/day, 5 days/week for $\leq$10.5 months.

Marked mortality occurred in exposed animals: 9/24 at the 315 mg/m^3 dose after a median of 44 exposures (exposure terminated at 135 days), 16/24 at the 630 mg/m^3 dose after a median of 10 exposures, 13/24 at the 1260 mg/m^3 dose after a median of three exposures, and 19/24 at the 2520 mg/m^3 dose also after a median of three exposures.

The guinea pigs exposed to the 315 mg/m^3 dose developed cirrhosis and hobnail surface alterations of the liver in 105 exposures. The authors concluded that survival of guinea pigs at higher doses was of insufficient duration to allow development of cirrhosis (Smyth et al., 1936). In addition, granular swelling was observed in adrenal glands of guinea pigs exposed to carbon tetrachloride at 315, 630 and 1260 mg/m^3 for 8, 7 and 17 exposures, respectively. Exposure to higher concentrations (1260 or 2520 mg/m^3) or continued exposure to lower concentrations resulted in marked damage to the sciatic nerves. Dense clumps of black granules (osmic acid stain) were observed paralleling the large majority of fibers.

Rhesus monkeys were also examined. They inhaled CCl_4 at concentrations of 320 and 1280 mg/m^3 for 8 hours/day, 5 days/week for 10.5 months (Smyth et al., 1936). Monkeys exposed to 1280 mg/m^3 showed an 8% less weight gain than the controls. At both 320 and 1280 mg/m^3, monkeys had livers with slight fatty degeneration following 8.7 months of exposure. The livers returned to normal 28 days post exposure. Two of four monkeys exposed to 1280 mg/m^3 for 8.7 and 10.5 months showed definite damage to the sciatic nerve.

In a chronic oral exposure study (Alumot et al., 1976), groups of 36 rats (18 male and 18 female littermates) were fed mash containing carbon tetrachloride at 0, 80 or 200 mg/kg of feed. The feed was stored in air-tight containers, assayed for carbon tetrachloride content, and consumed

soon after removal to feeding troughs. The authors calculated that the 200 mg/kg of feed represented a daily dose of 10 to 18 mg/kg bw. During this 2-year study, several parameters were measured. Up to 13 weeks no effects were noticed on body weight gain. Throughout the study measurements of male and female fecundity remained essentially normal. After 2 years, the surviving animals were killed. In these animals, serum values for glucose, protein, albumin, urea, uric acid, cholesterol, SGOT and SGPT in the treated animals did not differ from those in controls. No fatty livers were detected in the treated animals. Thus, the authors found no biochemical, histopathologic, reproductive or other abnormalities attributable to carbon tetrachloride exposure. However, interpretation of the results was complicated by the widespread incidence of chronic respiratory disease in the animals which started at about 14 months into the experiment. More than half the animals were dead at 21 months, although at 18 months the survival ranged from 61 to 89%. Although the authors indicated that 10 to 18 mg/kg bw (200 mg/kg of feed) is a no-observable-adverse-effect level (NOAEL) of carbon tetrachloride over 2 years, this conclusion may be questioned because of the chronic respiratory infection and hence poor survival of the animals in the latter part of the experiment. Yet, it may be inferred from these results that a level of 10 to 18 mg/kg bw/day over a 1-year period caused no observable adverse effects.

A chronic inhalation study by Adams et al. (1952) also presented incomplete data. No numbers of animals tested, surviving, or affected are given and it is not possible to determine what measurements were made at different exposures. The only conclusion possible is that at some exposures ranging from 32.5 to 2600 mg/m^3, 7 hours/day, 5 days/week for 258 days, some liver damage occurred in rats and rabbits.

In a study previously discussed (Merkureva et al., 1979), the authors also examined the effect of chronic CCl_4 inhalation. Long-term exposure to 300 mg CCl_4/m^3 caused a considerable increase in the DNA-synthesizing connective tissue cells (Merkureva et al., 1979).

Rotenberg (1978) examined the dynamics of liver bioenergetic system responses following the chronic exposure of rats to small concentrations of CCl_4 in air (14 mg CCl_4/m^3, 5 hours/day, 5 days/week for 5 months). As stated by the authors, the exposure caused phasic changes in hepatic energy-producing processes as evidenced by alterations in respiratory rate, phosphorylation and sensitivity of respiratory enzymes to respiration inhibitors (Rotenberg, 1978).

8.2. HUMANS

Many poisonings have resulted from the accidental or suicidal ingestion of CCl_4 or from its medicinal use as an anthelmintic. For its medicinal use, the therapeutic dose recommended for adults was 2 to 3 mℓ in capsule form and 0.13 mℓ/year for infants and children up to 15 years of age (von Oettingen, 1964). As emphasized by von Oettingen (1964), such doses, which are followed by doses of Epsom salts, have caused toxic effects only exceptionally. Horrocks (1934) reported one fatality from its medicinal use. The vast majority of poisonings, however, have resulted from the inhalation of its vapors when used as a solvent or dry cleaning agent (von Oettingen, 1964). Still other poisonings have been the result of dermal exposures through the use of CCl_4 in shampoos (NIOSH, 1975). Finally, some have resulted from its use in fire extinguishers (Dudley, 1935).

Norwood et al. (1950) reported the occurrence of 2 fatalities, 1 near fatality, 4 poisonings requiring hospitalization, and 51 mild industrial poisonings in two communities over a period of 1 year. In 1935, Smyth

(1935) noted 28 fatalities, 14 of which resulted from the ingestion of CCl_4; 120 acute and subchronic poisonings; and 7 cases of chronic poisoning. By 1964, an additional 28 poisonings resulting from CCl_4 ingestion (including 10 fatalities) and 202 cases from inhalation (including 29 fatalities) were reported. The actual incidence of such poisonings is doubtless much greater, since many poisonings are not attributed to CCl_4 and others are not published in the medical literature (von Oettingen, 1964). Since 1964, there have been additional poisonings and case reports (Bagnasco et al., 1978; Bonitenko and Bruk, 1979; Shimanko et al., 1979; Campbell et al., 1980); however, the total number has not been compiled.

8.2.1. Case Reports.

8.2.1.1. ACUTE — Oral poisonings from acute exposure to CCl_4 have occurred to a great extent, as reported by a number of authors (Docherty and Burgess, 1922; Beattie, et al. 1944; NIOSH, 1975; Kirkpatrick and Sutherland, 1956; Dawoorn et al., 1961). A summary of the symptoms of such oral poisoning is given below (von Oettingen, 1964). Following ingestion of CCl_4, the patient experiences a burning sensation in the mouth, esophagus, and stomach. Depending upon the dose, this is sooner or later complicated by abdominal pain, nausea, and vomiting. Some patients develop hiccoughs. The tongue is coated. These symptoms are soon followed by diarrhea, which later may be followed by constipation and occasionally by gastric and intestinal hemorrhages which, in rare cases, may also be seen in the mouth and pharynx. Again, depending upon the dose along with other factors, the patient becomes jaundiced, the liver becomes enlarged and tender; this may be associated with ascites and generalized edema. Soon after the ingestion, the patient feels dizzy, may suffer from headache and become confused, semiconscious and delirious. The patient may become restless and develop

choreatic movements. Finally, consciousness is lost and the patient passes into coma. Some patients complain of visual disturbances and edema of the eyelids and develop hemorrhages of the sclerae. In severe cases, circulatory disturbances may develop, characterized by lowered or increased blood pressure, thin and rapid pulse, and signs of congestive heart failure with cyanosis.

A fatality attributed to ingestion of carbon tetrachloride was reported by Smetana (1939). The victim, a photographer described as having "a history of chronic alcoholism," died 10 days after consuming an unknown amount of "some fluid containing carbon tetrachloride." He presented symptoms including nausea, vomiting, jaundice, anuria and semistupor. In the final clinical diagnosis, death was attributed to carbon tetrachloride poisoning.

A case of attempted suicide by ingestion of carbon tetrachloride was reported by Stewart et al. (1963). The victim, a 29-year-old female who ingested one pint of a carbon tetrachloride:methanol solution (2:1), experienced ringing in the ears immediately after ingestion and lost consciousness. She was hospitalized for 3 weeks. Three hours after ingestion, carbon tetrachloride in the exhaled breath and blood was confirmed by infrared analysis. The exhaled breath was then monitored throughout the hospitalization; CCl_4 was reported to decrease exponentially. Because of the toxicity of the methanol and the possibility of synergistic reactions with the carbon tetrachloride, hemodialysis was performed soon after admission. Mannitol solution was given by continuous intravenous infusion. Clinical laboratory analyses during hospitalization showed some elevation of SGOT, which reached a maximum of 75 units on day 6, and an elevation of urinary urobilinogen to a maximum of 7.8 Ehrlich units on day 10. Other laboratory findings included elevation of serum iron and depression of serum protein con-

centration and albumin fractions. The retention time of bromosulfopnthalein was increased. These findings were interpreted as evidence of minimal hepatocellular injury. Acute renal dysfunction was not observed; the authors credited the mannitol treatment with preventing renal damage.

Lamson and Minot (1928) studied the lethal effects of carbon tetrachloride on patients receiving carbon tetrachloride and magnesium sulfate orally as a treatment for hookworms. The authors reported the treatment of thousands of patients with a single dose of 2.5 to 15 mℓ carbon tetrachloride without ill effects. One man was reported to have safely ingested 40 mℓ of carbon tetrachloride. However, an "extremely small" population of adults died after receiving 1.5 mℓ of carbon tetrachloride; doses of 0.18 to 0.92 mℓ were reported to be fatal to children. Susceptibility in adults was correlated with alcoholic intake (chronic alcoholism or exposure to alcohol shortly after treatment), the presence of ascarid worms, and the intake of foods, particularly of high fatty content.

There are few postmortem reports on pathological changes in patients after the ingestion of CCl_4. McMahon and Weiss (1929) examined a 34-year-old male alcoholic who died five days after drinking one ounce of CCl_4. They discovered some reddish-brown fluid in the abdominal cavity, early atheromatous lesions in the heart, congested and edematous lungs with scattered petechial hemorrhages, enlarged and congested kidneys, marked erosion of the esophagus, and a congested and enlarged fatty liver.

Cases of acute toxicity associated with CCl_4 inhalation by humans have been more numerous. Bilateral peripheral constriction of the ocular color fields, resulting in symptoms of toxic amblyopia in three males, was attributed to the inhalation of carbon tetrachloride vapors (Wirtschafter, 1933).

Five male employees of dry cleaning establishments who had been exposed to carbon tetrachloride (of unknown concentration) from 8 to 10 hours daily for 1 to 6 months were examined. Two men also had signs of conjunctivitis. Three of the men complained of visual disturbances characterized by blurred vision or spots before the eyes. Wirtschafter concluded that toxic amblyopia may result from exposure to carbon tetrachloride vapor.

One fatality occurred in two cases of carbon tetrachloride poisoning reported by Smetana (1939). In the fatality, a dry cleaner and interior decorator described as being "a steady and heavy drinker" was exposed for several hours to carbon tetrachloride vapors (concentration unknown) during work. Upon returning from work, he noted dyspnea. Several hours after the exposure, headache, dizziness and malaise developed, accompanied by nausea and repeated vomiting that persisted for several days. The patient also suffered labored breathing and cough with bloody sputum before he died 9 days after exposure.

The second inhalation case reported by Smetana was a housemaid also described as having a history of chronic alcoholism. Three days before hospitalization, the patient cleaned dresses with carbon tetrachloride for 3 hours in a poorly ventilated room. Soon after exposure, she began to vomit. She suffered symptoms similar to those described for the other case. After approximately 1.5 months of hospitalization, this patient was released; her condition several weeks later was described as "much improved."

Seven of the cases of carbon tetrachloride poisoning reported by Norwood et al. (1950) resulted from both occupational and nonoccupational inhalation exposures. In the three cases described as "severe" poisonings, there was a history of chronic alcoholism; two fatalities occurred in this group. In one fatal case, the victim had been exposed for about 25 minutes to an

atmosphere containing carbon tetrachloride at an estimated 1575 mg/m^3 (this estimate was made by duplicating the conditions). Histopathological examination of liver and kidney tissue from the fatalities revealed liver necrosis and degeneration of the renal tubules. The four remaining cases were characterized as "mild industrial" exposures. After exposure to carbon tetrachloride, all subjects suffered varied symptoms including nausea, vomiting, diarrhea, headache, muscular ache, pain or numbness, labored breathing and dizziness.

In another case, a 31-year-old janitor suffered malaise, back and lower abdominal pain, nausea and vomiting the morning after working for 5 hours in a closed room with carbon tetrachloride (concentration unknown) (Kittleson and Borden, 1956). He reportedly consumed two bottles of beer during the exposure period. The patient required 2 months of hospitalization for treatment of acute renal insufficiency as a result of carbon tetrachloride intoxication.

Elevated SGOT activities with concomitant liver changes were reported in two men occupationally exposed to unreported concentrations of carbon tetrachloride (Lachnit and Pietschmann, 1960). One became ill after exposure to carbon tetrachloride for 3 hours in a relatively well-ventilated room. He was hospitalized 3 days after exposure. His liver was slightly enlarged, with the SGOT value elevated by 6000 units. This value rapidly decreased and by the 10th day had returned to normal. A biopsy of the liver taken on the 8th day showed necrosis in the centers of the lobuli, but the surrounding tissue was undamaged. An additional needle biopsy of the liver taken on the 28th day showed that the cells had almost returned to normal. In the second case a male similarly exposed to carbon tetrachloride entered the

hospital 12 days after exposure. The SGOT had increased to 80 units. A liver needle biopsy on the 22nd day showed only moderate changes, some of a degenerative nature.

In a chemical packing plant, use of carbon tetrachloride by two workers for equipment cleaning as a substitute for the customarily used acetone, resulted in the hospitalization of 4 of 43 workers at the plant (Folland et al., 1976). Ten additional workers became ill. Eight of the 43 workers fell ill within 12 hours following the start of the 2-hour exposure; six others followed within the next 36 hours. The four hospitalized workers showed evidence of severe disruption of liver function: one case had an SGOT level of 13,390 units. All patients recovered within 90 days. All hospitalized workers, as well as most of the others taken ill, had worked near a bottle-filling operation for isopropyl alcohol at the northern end of the plant, adjacent to the carbon tetrachloride cleaning area.

Carbon tetrachloride concentrations at the time of exposure were not ascertained; acetone was normally used for cleaning. Isopropyl alcohol concentrations at the northern end of the plant averaged 2624 mg/m^3. Acetone in alveolar air samples of workers in the northern area averaged 121.6 mg/m^3. The authors ascribed the toxic episode to carbon tetrachloride toxicity potentiated by isopropyl alcohol. Because carbon tetrachloride concentrations were unknown and isopropyl alcohol (and possibly other chemicals) were present, the health effects reported in this study cannot be attributed to carbon tetrachloride exposure alone.

Some of these reports and others (Davis, 1934; Stewart et al., 1961; Smith, 1950; NIOSH, 1975; von Oettingen, 1964) indicate that with single exposure to low concentrations, there is considerable variation in symptoms among different persons and that the acute toxicity is relatively low in

contrast to that with repeated exposure. Cases in which exposure is light may be restricted to such symptoms as moderate irritation of the eyes, moderate dizziness and headache, which disappear promptly upon discontinuation of the exposure.

The immediate effects from acute inhalation exposure to higher concentrations of CCl_4 consist of the same symptoms as described above, but in addition the patient may become nauseated and suffer from loss of appetite, mental confusion, agitation and the feeling of suffocation. In severe cases, the patient may lose consciousness and develop fever and chills. The tongue may be furred and the patient may suffer from vomiting with bloody or oile-stained vomitus which may last for days, colicky pain and diarrhea with liquid brown-black or bloody stools (von Oettingen, 1964). This ten- dency for hemorrhages may also result in bleeding from the gums and nose, hemorrhages under the skin and macular papular rashes. The colicky pain may be associated with a marked abdominal resistance simulating the "acute abdomen" and thus has been mistaken for appendicitis and peptic ulcer. Following such an acute episode, the patient feels tired and weak and frequently suffers from headache. The patient may develop muscular twitchings and epileptic convulsions. In a few instances, paralysis (hemiplegia) and polyneuritis have been reported (von Oettingen, 1964).

In more severe innalation poisoning blood pressure may be lowered, but as renal complications develop, the blood pressure is usually elevated and the cardiac output decreases because of increased peripheral resistance. The pulse may be accelerated. In the case of severe inhalation poisoning, the patient may collapse. Electrocardiograms have shown changes characteristic of myocardial injury characterized by sinus bradycardia and followed by auriculoventricular arrhythmia, auricular fiorillation and sinus arrhythmias (von Oettingen, 1964).

Depending upon the condition of the patient, respiration may be normal, rapid and shallow, or slow and labored. The latter is evident especially if circulatory failure is imminent and pulmonary edema develops. Thompson (1946) found that early roentgenograms of the lungs may show pulmonary involvement.

In most instances after the severe inhalation exposure, the patient develops signs of liver injury within a few days. The patient becomes jaundiced and the liver becomes enlarged and tender. This is toxic hepatitis, which may pass into yellow atrophy and, in more protracted cases, eventually into cirrhosis of the liver. In the early stages of liver injury, even before a marked enlargement occurs and while liver function tests such as the cephalin-flocculation test are still normal, the SGOT level may be markedly elevated (von Oettingen, 1964).

As signs of liver injury develop, and sometimes in their absence, injury of the kidneys may dominate the clinical picture and be responsible for early death (von Oettingen, 1964). Kittleson and Borden (1956) characterized renal failure by three phases. The first phase is characterized by polyuria and nocturia, which may result in severe dehydration, followed by oliguria and finally by diuresis. The renal injury may result in acute nephritis with albumin, red and white cells, and casts in the urine. In some patients, the presence of acetone and sugar in the urine has been reported. The oliguria may be associated with increased blood levels of potassium, indican, phenol, cresol, creatinine and urea; the latter may result in uremia. In other instances, the injury may consist of necrotizing nephrosis with comparatively little change in the urinary composition. The renal blood flow and glomerular filtration rate are decreased; and the former seems to be mainly responsible for the maintenance of oliguria, being the

sequela rather than the cause of renal failure (von Oettingen, 1964). During the early stage of oliguria, abnormal tubular back diffusion of the filtrate may play an important role. Oliguria may develop as early as 24 hours or 3 to 4 days after onset of the poisoning and may persist for 12 to 14 days and even longer (von Oettingen, 1964).

In the early stages after severe inhalation poisoning and during the period of polyuria, the blood may show some polycythemia, but later this may be followed by anemia and lowering of the hematocrit levels because of hemodilution. The most important changes in the blood are, however, related to the biochemical composition of the blood which reflects the renal and hepatic injury. As soon as the renal injury develops, the nonprotein nitrogen and urea nitrogen levels in the blood are increased and may reach extremely high values. The creatinine, indican, phenol, and cresol levels may also be increased. In the case of liver injury, as related to the blood, the icteric index is usually increased, and the levels of sugar and phospolipids, along with the ratio of chloesterol esters over chloestrol, are reduced. The prothrombin time and the fibrinogen content may be reduced, resulting in an increased clotting time. The chloride level is frequently lowered by hemodilution or severe vomiting, and the potassium level may be elevated. This increase in potassium may contribute to ventricular fibrillation or cardiac arrest (von Oettingen, 1964).

In addition to constriction of the visual field and toxic amblyopia (Wirtschafter, 1933), carbon tetrachloride poisoning can also result in blurred and double vision. Conjunctival hemorrhages are common. Retinal hemorrhages and exceptional cases of the degeneration of the optic nerve have been reported (von Oettingen, 1964).

Conway and Hoven (1946) report that after ingestion of CCl_4, certain electrocardiographic changes may be observed indicating degenerative processes in the heart muscle, such as sinus bradycardia, followed by auriculoventricular rhythm, auricular fibrillation, and sinus arrhythmias. The respiration varies with the condition of the patient. If the patient is in collapse, it will be rapid and shallow; if the patient is comatose, it may be labored and dyspneic, and pulmonary edema and hemorrhages may develop. Eventually, disturbances develop characterized by polyuria and followed by oliguria which may pass into anuria. The urine of such patients is rich in albumin and may contain blood and casts. If the liver is damaged, the urine will contain urobilinogen, urobilin, and bile pigments. The nonprotein nitrogen level in the blood will be increased and the patient may suffer from hypoprothrombinemia, hypochloremia, and signs of acidosis. Death may ensue after 8 hours, or 3, 5 or 10 days, and sometimes later.

Two cases involving the pancreas following inhalation or an acute exposure of CCl_4 were reported by Jahnke (1953). Both patients became listless and developed hepatic and circulatory disturbances and sensitivity of the pancreas to pressure. Such disturbances were long term and had not completely subsided after 10 months.

Guild et al. (1958) report on 20 cases of CCl_4 intoxication which resulted in renal damage manifested by anuria. Four of these were the result of ingestion. Exposures varied: one drank 15 cc; one drank 4 ounces; 2 drank small amounts, but were also consuming alcohol (see Chapter 12). The remaining 16 cases inhaled CCl_4. Exposure times ranged from 30 minutes to 11 hours. Two of the four that ingested the CCl_4 died whereas three died of the 16 exposed through inhalation.

Severe liver involvement was seen in all four of those ingesting CCl_4. Eleven of the 16 who inhaled CCl_4 had "moderate to severe" liver involvement whereas the remaining 5 had no liver involvement (Guilo et al., 1958). These discrepancies can be attributed in part to the variability in exposure amounts and duration; however, this cannot be resolved from the report. The role of alcohol consumption also cannot be defined; however, this report does lend some support to the research discussed in Chapter 12.

Stevens and Forster (1953) report on the neurological effects of CCl_4. Fifteen cases of CCl_4 intoxication with varying amounts and routes of exposure are discussed. Thirteen occurred in the home and two occurred in commercial cleaning plants. Of the 13 cases occurring at home, 2 were children (the youngest being 9 months old) who drank the fluid. Two were adults who accidentally drank it, and the rest were exposed via inhalation. There were five deaths. Eleven of the 13 were alchoholic. Four of the deaths occurred in alcoholics; for the fifth, no history was available (Stevens and Forster, 1953). Seven of the fifteen experienced neurological symptoms. Of these, two children who ingested the chemical displayed stupor, drowsiness or unconsciousness. The adults displayed headache, vertigo, weakness, blurred vision, lethargy and coma.

8.2.1.2. **SUBCHRONIC/CHRONIC** -- Subchronic/chronic human exposures to carbon tetrachloride are nearly always by inhalation. Headache, fatigue, dizziness, nausea, and vomiting occur at substantially lower concentrations in subchronic and chronic exposures than in acute exposure (Elkins, 1942; Kazantzis and Bomford, 1960). As in acute exposures fatty and necrotic livers are the common pathological findings (Gray, 1947; Dellian and Wittgens, 1962; Barnes and Jones, 1967). In some cases, renal injury is the major

finding. Renal tubular necrosis has been reported both with and without concomitant liver disease (Hamourger et al., 1958; Richet et al., 1959; von Oettingen, 1964).

CCl_4 poisoning as a result of chronic inhalation of low dose exposures have been reported by Butsch (1932), Wirtschafter (1933), Straus (1954), von Oettingen (1964) and others. The clinical picture after chronic CCl_4 exposure is much less characteristic than that after acute exposure. von Oettingen (1964) has reviewed the symptoms. With chronic exposure, patients may complain of fatigue, lassitude, giddiness, anxiety, and headache. They suffer from paresthesias and muscular twitchings and show increased reflex excitability. They may be moderately jaundiced, have a tendency to hypoglycemia, and biopsy specimens of the liver may show fatty infiltration. Patients may complain of lack of appetite, nausea, and occasionally diarrhea. In some instances, the blood pressure is lowered which is accompanied by pain in the cardiac region and mild anemia. Other patients develop pain in the kidney region, dysuria and slight nocturia and have urine containing small amounts of albumin and a few red blood cells. Burning of the eyes and, in a few instances, blurred vision are frequent complaints of those exposed. If these symptoms are not pronounced or of long standing, recovery usually takes place upon discontinuation of the exposure if the proper treatment is received (von Oettingen, 1964).

Straus (1954) suggested a possible causal relationship between carbon tetrachloride exposure and aplastic anemia. Three males had been exposed via inhalation and dermal absorption to carbon tetrachloride at unknown concentrations for 2 months to 3 years. Autopsy findings included hypoplasia of the bone marrow. However, a causal relationship between carbon tetrachloride and aplastic anemia suspected by the author in these cases is not

supported adequately. One of the men had also been exposed to kerosene for 3 years. Another was an auto mechanic who worked in a garage. The occupation of the third was not specified although his exposure to carbon tetrachloride was occupationally related. Thus the effects of other chemicals cannot be discounted. The autopsy findings of two of the patients included no liver or kidney damage of the type that would be expected in carbon tetrachloride poisoning. In one case the liver was reported to have periportal fibrosis and fatty infiltration. These findings were attributed to toxic hepatitis which was considered to be the result of carbon tetrachloride poisoning. The information reported in these case studies tends not to substantiate the author's suggestion that the patients' illnesses may have been caused by carbon tetrachloride.

Postmortem reports on pathological changes in patients after inhalation of CCl_4 are generally limited to findings in the liver and kidneys. The liver may show nutmeg appearance and fatty degeneration even in the absence of clinical signs and symptoms of liver injury. In other instances, centrilobular necrosis and hemorrhages with infiltration of leukocytes and histiocytes and collapse of the lobules with condensation of the reticular framework within these areas are seen. After chronic exposure, there may be evidence of regeneration of the liver cells (von Oettingen, 1964).

Postmortem changes in the kidney are characterized by nephrosis, by a distention of Bowman's capsule with albuminous precipitates, and by swelling of the lining cells. The cells of the convoluted tubules may be swollen and vacuolated; later, degenerative changes may be seen in Henle's loops, associated with granular, hyaline, and cellular casts in the tubules. After chronic exposure, regenerative changes may be visible in these regions. In other cases, the kidneys may offer the picture of acute hemorrhagic nephritis (von Oettingen, 1964).

Other postmortem organ changes are less characteristic for CCl_4 poisoning and vary considerably with the clinical picture. Some changes may occur that are a direct result of the changes occurring in the primary target organs of CCl_4. Stasis of various organs is the most outstanding feature of cardiac failure. The brain and lungs may be edematous. The intestines may be hyperemic and covered with numerous petechial hemorrhages, and the spleen may be enlarged and hyperemic. Occasionally the adrenal glands may show degenerative changes of the cortex, and the heart may undergo toxic myocarditis (von Oettingen, 1964).

8.2.2. Controlled/Clinical Studies. Human volunteers were exposed to known concentrations of carbon tetrachloride vapor in an effort to correlate physiological and/or biochemical changes to the magnitude of exposure (Stewart et al., 1961). Eight healthy male volunteers were exposed to carbon tetrachloride vapors in a series of three separate experiments performed 1 month apart. Prior to exposure, data on blood pressure, SGOT and urinary urobilinogen were obtained for each subject. Samples of pre-exposure exhaled breath, urine and blood showed no detectable carbon tetrachloride. The volunteers were seated in a closed room (11 x 12 x 7.5 feet) where 99% pure carbon tetrachloride was poured into a dish and covered with a towel. An exhaust system grill and door were closed during the experiment but an air supply grill was left open. A fan circulated air across the dish. Carbon tetrachloride ambient concentrations were monitored with a Davis halide meter and an infrared spectrometer. The carbon tetrachloride concentration ranges and exposure times are given in Table 8-6.

Carbon tetrachloride was detected in exhaled breath in all three experiments. Graphs showed an exponential decrease in concentration of carbon tetrachloride versus time. The exact values were not given.

TABLE 8-6

Exposure Times and Concentrations of Carbon Tetrachloride Vapor In a Controlled Human Study*

Experiment	Average Concentration, Time-weighted (mg/m^3)	Concentration Range (mg/m^3)	Exposure (minutes)
1	309	192-548	70
2	69	63-88	180
3	63	57-88	180

*Source: Stewart et al., 1961

The serum iron showed an initial decrease in 3 of 6 subjects at the 309 mg/m^3 exposure level but had returned to normal in two of these subjects 68 hours after exposure. The remaining subject showed a 31% depression in serum iron at 68 hours, but the value was within the normal range. Serum iron was not analyzed in the other two experiments. Of the six subjects exposed to carbon tetrachloride at 309 mg/m^3, the serum transaminase level was slightly elevated in some and depressed in others, but remained within the normal range. Carbon tetrachloride was not detected in the blood or urine at any exposure time or dose, but the analytical technique used (infrared method) was not a sensitive one. The authors concluded that no ill effects were observed from exposure to carbon tetrachloride at 63 mg/m^3 for 180 minutes, although the small changes in serum iron at the 309 mg/m^3 dose might have been an indication of liver insult.

A survey conducted by Kazantis and Bomford (1960) was the result of the complaints of a worker in a factory processing raw quartz. Intermittently for 2 years, he experienced anorexia, nausea and occasional vomiting with abdominal discomfort. He noticed that his symptoms or dyspepsia worsened during the work week but got better on the weekends. The investigators interviewed 17 of the 18 employees working in the same area taking medical and occupational histories. Their ages ranged from 16 to 54. Fifteen of the 17 complained of symptoms similar to the initial case, primarily nausea, anorexia, vomiting, flatulence, epigastric discomfort and depression. These symptoms had been occurring among the workers from 1 week to 2 years. Environmental monitoring measured CCl_4 atmospheric concentrations of 292.5 to 650 mg/m^3. Once the cause of the high concentration was found, changes were made in the processing procedure. Within 1 week following these changes, the symptoms disappeared in all workers. Follow-up for 6 months revealed no recurrences.

Direct application of CCl_4 to human skin causes a burning and stinging sensation within 5 minutes. The maximum pain is reached 6 minutes later and is associated with erythema, hyperemia, and wheal formation, later followed by vesication (Oettel, 1936).

The absorption of carbon tetrachloride through human skin was measured by immersion of the thumbs of three male and female volunteers in a sample of this compound for 30 minutes (Stewart and Dodd, 1964). The carbon tetrachloride was analyzed by infrared spectroscopy, and no impurities were detected. Sequential sensations of burning and cooling were experienced by all volunteers during the immersion. Burning ceased about 10 minutes after removal from the solvent. The thumbs of all volunteers appeared scaly and red, a condition that improved within several hours after exposure. Carbon tetrachloride was detected in the alveolar air of each subject within 10 minutes of immersion of their thumbs. The concentration in the expired breath rose continuously to a maximum of 4.0 mg/m^3 10 to 30 minutes after the exposure period ended, and then decreased exponentially. The mean concentration of carbon tetrachloride 2 hours after the end of exposure was 2.0 mg/m^3; at 5 hours after exposure, the alveolar air concentration was still >0.6 mg/m^3. The authors concluded that carbon tetrachloride could be absorbed through the skin in toxic quantities.

Hall (1921a,b) demonstrated the effectiveness of CCl_4 as a vermicidal agent in treatment of hookworm infestations. The usage of CCl_4 in this capacity stimulated considerable research efforts to investigate the pharmacologic and physiologic effects of CCl_4 on humans (NIOSH, 1975). The effects of oral doses of CCl_4 as a human anthelmintic, administered to condemned prisoners in Ceylon has been reported (Docherty and Burgess, 1922;

Docherty and Nicholls, 1923). Three of the prisoners received 4 mℓ CCl_4, two received 5 mℓ CCl_4, and one received 5 mℓ plus an additional 3 mℓ two weeks after the first dose. Execution of the prisoners occurred 3 to 15 days after the CCl_4 administration. Autopsies were performed and the findings varied. The livers of some showed no major microscopic or macroscopic changes whereas the livers of other showed marked fatty degeneration. From such data, a dose-response relationship would be difficult to determine (NIOSH, 1975).

A cross-sectional epidemiologic study (Sonich et al., 1981) examined the effect upon persons exposed to CCl_4 through their drinking water. Seventy tons of CCl_4 were spilled in the Kanawha and Ohio Rivers in 1977. Measurements of raw water revealed maximum concentrations of 0.340 mg/ℓ. Twenty-one cities situated along the river were involved in the study. These cities represent areas that draw their drinking water directly from the river and/or areas that draw their drinking water from sources not influenced by the quality of the river water. Measurements at each of the four major cities along the river showed a decrease in the CCl_4 concentration in the river with the number of river miles from the spill: Huntington, West Virginia - 0.210 mg/ℓ; Cincinnati, Ohio - 0.180 mg/ℓ; Louisville, Kentucky - 0.110 mg/ℓ; Evansville, Indiana - 0.060 mg/ℓ. Finished drinking water in Cincinnati contained a peak level of 0.087 mg/ℓ. A control period was identified in 1976 when there were non-detectable to trace amounts of CCl_4 in the river as indicated by monitoring efforts at Cincinnati. By using river volumes and flow rates, periods of high exposure (1977) and low exposure (1976) to CCl_4 were estimated for each city along the river. A total of 35 hospitals in these cities provided medical data on patients admitted during these estimated time periods. The results of

routine tests measuring serum chemistries reflecting liver and kidney function along with basic epidemiologic information were abstracted from approximately 6000 medical records. It is hypothesized that CCl_4 would cause a rise in some or all of these serum chemistries. The data were categorically analyzed to test for a dose/response relationship. In this capacity, the ratio of the odds ratios (ROR) for each serum chemistry for each city group were computed as $ROR = OR_{77}/OR_{76}$ where

$$\text{odds ratio}_{76} = (OR_{76}) = \frac{\text{the odds of having an elevated test result while drinking Ohio River water in 1976}}{\text{the odds of having an elevated test result while drinking water in 1976 not affected by the quality of the Ohio River}}$$

and

$$\text{odds ratio}_{77} = (OR_{77}) = \frac{\text{the odds of having an elevated test result while drinking Ohio River water in 1977}}{\text{the odds of having an elevated test result while drinking water in 1977 not affected by the quality of the Ohio River.}}$$

The results obtained for creatinine show a positive and statistically significant ($p<0.05$) dose/response relationship between the CCl_4 exposure and the ROR or frequency of elevated levels of serum creatinine in exposed patients in relation to the controls as determined by a test for linearity of trend. Other parameters analyzed were alkaline phosphatase, total bilirubin, blood urea nitrogen, lactic dehydrogenase, SGOT and γ-glutamyl transpeptidase. No similar results were found for these parameters.

8.3. MECHANISMS OF TOXICITY

The toxicity of carbon tetrachloride to an organism depends upon the ability of the organism to metabolize the compound; unmetabolized carbon tetrachloride does not appear to be significantly toxic (Recknagel and Glende, 1973). In mammals, as discussed in Chapter 7, carbon tetrachloride is thought to be metabolized in the endoplasmic reticulum of the liver by the mixed-function oxidase system of enzymes. A reaction sequence proposed

in the literature for carbon tetrachloride metabolism is outlined in Figure 7-1. Two free radicals have been postulated as metabolic intermediates: the trichloromethyl radical and the chlorine radical. The toxicity of carbon tetrachloride has been attributed to subsequent reactions of the trichloromethyl radical. These reactions include formation of carbonyl chloride (phosgene), dimerization to hexachloroethane, free radical binding to protein, and lipid peroxidation. In this chapter each of these proposed pathways will be presented in conjunction with the toxic effects attributed to it.

8.3.1. **Formation of Carbonyl Chloride (Phosgene).** From the results of an in vitro study of carbon tetrachloride metabolism, Shan et al. (1979) postulated the formation of carbonyl chloride from the trichloromethyl radical. As previously discussed, the authors incubated L-cysteine and [^{14}C]carbon tetrachloride with rat liver homogenate and looked for the formation of 2-oxothiozolidine-4-carboxylic acid. This compound is formed from the reaction of L-cysteine and carbonyl chloride. Analysis of the metabolic products by mass spectroscopy showed a fragmentation pattern consistent with 2-oxothiozolidine-4-carboxylic acid. The authors inferred from these analytical data that carbonyl chloride was formed as a metabolic product of carbon tetrachloride. Although carbonyl chloride (phosgene) is not reported to be a carcinogen, the authors pointed out that the compound is highly toxic and that the reactive chlorines could react with macromolecules in ways similar to alkylating agents.

8.3.2. **Dimerization to Hexachloroethane.** Hexachloroethane has been identified as a metabolite of carbon tetrachloride by Fowler (1969). The formation of this compound is believed to take place by the dimerization of the trichloromethyl radical. Although hexachloroethane is a hepatotoxin, its

toxicity is less than that seen in carbon tetrachloride poisoning. Therefore, other mechanisms probably account for the severe toxicity of carbon tetrachloride.

8.3.3. Free Radical Binding to Proteins. Free radical binding to proteins has been postulated as one cause of toxicity associated with carbon tetrachloride (Recknagel and Glende, 1973). The binding was reported to involve reactions with cellular proteins, particularly those with sulfhydryl groups. Delvillarruel et al. (1977) found a good qualitative correlation between intensity of CCl_4 activation and P-450 content of various organs in male, virgin female, or pregnant female rats. No correlation was found between CCl_4 activation and cytochrome c reductase activity. The authors state that their results suggest that P-450 is involved in CCl_4 activation and that irreversible binding of CCl_4 metabolites to cellular components, rather than lipid peroxidation, is responsible for some biochemical and/or ultrastructural lesions reported in different tissues. In another study, [^{14}C]carbon tetrachloride has been observed to bind irreversibly to rabbit microsomal proteins at a rate of approximately 20 nmole/mg protein/hour (Uehleke and Werner, 1975). Binding of carbon tetrachloride (or its metabolites) to hepatic macromolecules was enhanced in the absence of oxygen, consistent with the proposal that the trichloromethyl radical is the reactive metabolite. Furthermore, the order of species susceptibility to liver necrosis from carbon tetrachloride more closely parallels the species order for [^{14}C]carbon tetrachloride binding to cellular components than the species order for lipid peroxidation (Diaz Gomez et al., 1975):

Effect	← Most susceptible ... Least susceptible →
Liver necrosis	mouse > guinea pig = hamster > rat > chicken
[^{14}C]CCl_4 binding	mouse = hamster > guinea pig > rat > chicken
Lipid peroxidation	rat > hamster = guinea pig > chicken = mouse

These authors also report that in mice liver necrosis proceeded 24 hours in the absence of lipid peroxidation.

Although it has been shown that [^{14}C] from carbon tetrachloride binds to proteins, the question of carbon tetrachloride binding to polynucleotides remains. The issue is important because of its implications for the mechanism of carbon tetrachloride carcinogenicity and mutagenicity. In the only experiment addressing this question, Uehleke and Werner (1975) incubated [^{14}C]carbon tetrachloride with either isolated liver microsomes (rat or mouse, species not identified) or with added soluble RNA. They reported no [^{14}C] binding to ribosomal RNA or exogenous RNA. Experimental details were not presented, but it appears possible to tentatively conclude that proteins -- rather than nucleic acids -- are the main sites of macromolecular carbon tetrachloride binding.

8.3.4. **Lipid Peroxidation.** A number of the hepatic effects resulting from carbon tetrachloride exposure, including the fatty liver syndrome, are believed to arise as a result of lipid perioxidation (Recknagel and Glende, 1973). The mechanism proposed for the peroxidation is presented below, followed by a discussion of the evidence that this biochemical sequence results in the hepatic lesions associated with carbon tetrachloride poisoning.

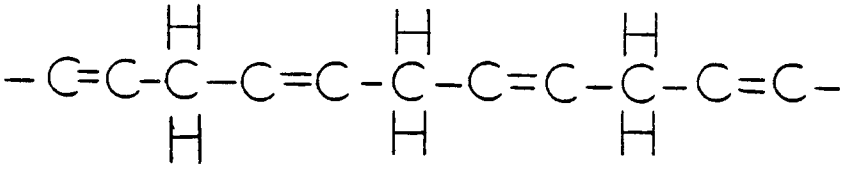
The first step in the reaction sequence proposed for lipid peroxidation is production of free radicals, especially the trichloromethyl free radical. The radical initiates a chain reaction by reacting with the hydrogen atom of a -CH_2-group in an unsaturated fatty acid, generating a fatty acid free radical. On reaction with molecular oxygen, the fatty acid free radical is converted into an unstable organic peroxide. The peroxide disintegrates in two fashions: (1) intramolecular cyclization to form malonic dialdehyde and two new free radicals, or (2) simple homolytic fission that also yields two

free radicals. This whole process occurs autocatalytically: each free radical gives rise to two new free radicals. Figure 8-1 summarizes this hypothesis (Recknagel and Glende, 1973).

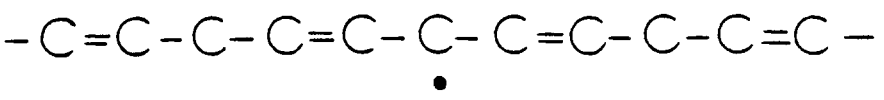
A number of indices have been used in in vivo and in vitro assays of lipid peroxidation: pentane and ethane levels in exhaled air (arising from fatty acid decomposition) and malonic dialdenyde concentrations in hepatocytes (arising from intramolecular cyclization). Pentane production in male rats increased by factors of 4.6, 13.2 and 26.4 over that in mineral oil controls within 30 minutes following i.p. administration of carbon tetrachloride doses of 160, 480 and 1440 mg/kg bw, respectively (Sagai and Tappel, 1979).

A mechanism for the pathogenesis of carbon tetrachloride-induced hepatic lesions based on lipid peroxidation has been proposed recently (Pasquali-Ronchetti et al., 1980). According to this hypothesis, lipid peroxidation is suggested to affect primarily unsaturated acyl chains of membrane phospholipids, resulting in breakage of the hydrocarbon and loss of phospholipids from the membrane. Lipid peroxidation would therefore produce progressive degenerative changes in the assembly of memoranous structures such as (rat) liver endoplasmic reticulum, or its in vitro counterpart, microsomes.

This hypotnesis is supported by studies showing that treatment with carbon tetracnloride produced lipid peroxidation in rat liver endoplasmic reticulum at a concentration of 0.5 mℓ/100 g bw (Pasquali-Ronchette et al., 1980), caused disintegration of endoplasmic reticulum in vitro within 10 minutes at a concentration of 636 mg/ℓ (Pasquali-Roncnetti et al., 1980), and was incorporated predominantly into liver phospholipids in rats (Table 8-7) (Ciccoli and Casini, 1978).

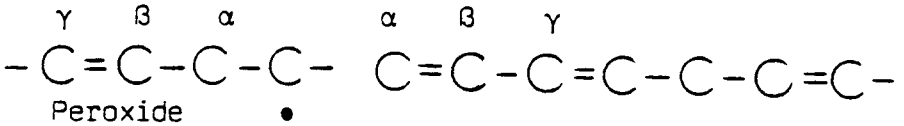


$HCCl_3$ • CCl_3 Trichlormethyl Free Radical



RESONANCE (All Possible Forms Not Shown.)

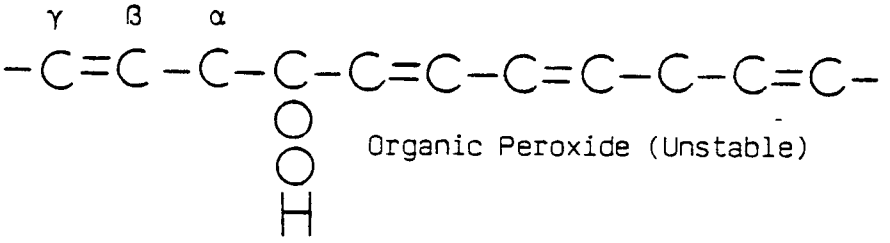
Organic Free Radical



Peroxide formation

O_2

diene conjugation, λ_{max}=233mu



Organic Peroxide (Unstable)

Intramolecular cyclization and decomposition to yield malonic dialdehyde and two new organic free radicals.

Decomposition to yield two free radicals. Eventual stable decomposition products highly organoleptic.

FIGURE 8-1

Free Radical Initiated, Autocatalytic Peroxidation of Polyenoic Long-Chain Fatty Acids

Source: Adapted from Recknagel and Glende, 1973

TABLE 8-7

Incorporation of ^{14}C from [^{14}C]Carbon Tetrachloride into Lipids of Various Rat Tissues[a,b]

Tissues	Total Lipids (dpm/mg)	Acetone Precipitate (phospholipids) (dpm/mg)	Acetone Supernatant Lipids (dpm/mg)
Liver (6)	112.6 ± 7.4	135.7 ± 14.6	53.3 ± 4.8
Intestinal mucosa	61.8 ± 7.5	69.5 ± 7.7	48.3 ± 8.4
Kidney (6)	23.4 ± 2.6	25.6 ± 3.9	11.4 ± 2.0
Adrenals[c]	8.0	22.0	2.4
Lung (5)	11.3 ± 1.8	15.2 ± 1.4	6.5 ± 0.8
Spleen (6)	8.7 ± 1.2	7.4 ± 2.3	4.0 ± 1.0
Testis (6)	6.3 ± 1.5	5.2 ± 1.4	2.3 ± 1.0
Brain (6)	3.3 ± 0.3	3.7 ± 0.3	0.7 ± 0.2
Heart (6)	2.2 ± 0.8	2.6 ± 1.4	0.8 ± 0.5
Skeletal muscle (6)	0.7 ± 0.1	2.9 ± 0.7	0.6 ± 0.2
Plasma[d]	5.1	trace	3.7

[a]Source: Adapted from Ciccoli and Casini, 1978

[b][^{14}C]CCl_4 dose: 4000 mg/kg bw (58.6 x 10^6 dpm). Values are expressed as means ± S.E.M. The number of rats is reported in parentheses.

[c]Eight pooled adrenals.

[d]Plasma of two animals.

dpm = disintegrations per minute

8.4. SUMMARY

Carbon tetrachloride is toxic to humans and animals following inhalation, ingestion or dermal administration. Acute, subchronic and chronic exposures primarily affect the central nervous system, liver and kidneys. Sporadic cases of ocular toxicity also occur following subchronic and chronic exposure to carbon tetrachloride vapor. However, these ocular signs do not correlate with exposure levels or other organ toxicities. Ingestion of alcohol appears to increase susceptibility to carbon tetrachloride toxicity, but the mechanisms are unknown.

8.4.1. Experimental Animal Data. The toxicity of carbon tetrachloride following acute inhalation, ingestion and dermal exposures has been reported for various species. Animals surviving acute doses of carbon tetrachloride developed liver damage and, in some cases, kidney damage. These injuries were dose related.

Subchronic/chronic studies of carbon tetrachloride exposure in rats, monkeys, rabbits, dogs and guinea pigs demonstrated liver, kidney, sciatic nerve, optic nerve and ocular muscle damage.

It has been observed that exposure to a higher concentration over a shorter period of time produces a greater effect upon the liver than exposure to a lower concentration over a longer period of time even though the product of time and concentration is equal in both cases.

8.4.2. Human Data. Considerable human exposure to carbon tetrachloride through inhalation has occurred through its use as an industrial solvent and dry cleaning fluid. Ingestion of carbon tetrachloride or of mixtures containing carbon tetrachloride has also been documented in various case reports. Ingestion has occurred under different circumstances by persons of diverse occupations and ages. These acute exposures have been followed by hepatoxic effects accompanied by acute nephrosis.

Hepatic necrosis and renal pathology appear to be characteristic effects of acute human exposure to carbon tetrachloride. If exposure is terminated, the liver shows regeneration in most cases. In cases of acute renal dysfunction, kidney function returns to normal after exposure to carbon tetrachloride is terminated and medical treatment is given.

In many of the case reports and older studies, the investigators present the data in narrative form. Although interesting, these type of data are not suitable to quantitative analysis since numbers are not adequately presented. Furthermore, there usually are a number of uncontrolled variables (alcohol intake, age, simultaneous exposures) or unknown variables (exposure amount) making it difficult to attribute the outcome solely to the CCl_4 exposure. The experimental human studies are not numerous, yet they are important since they can either support or challenge the experimental animal studies and can aid qualitatively in the extrapolations from animals to humans.

8.4.3. Mechanisms of Toxicity. The chemical pathology of CCl_4 liver injury is generally viewed as an example of lethal cleavage, where the CCl_3-Cl bond is split in the mixed function oxidase system of hepatocytes. Two major sequences of this cleavage have been suggested; both views presume the formation of free radicals from the homolytic cleavage of the CCl_3-Cl bond (i.e., $CCl_3\cdot$ and $Cl\cdot$). One sequence entails the direct attack (via alkylation) by free radicals on cellular constituents, notably protein sulfhydryl groups. The second sequence involves the abstraction of a hydrogen atom by the free trichloromethyl radical from a long-chain fatty acid to form chloroform and a fatty acid free radical. Molecular oxygen, because of its triplet ground state, binds with the unpaired election on the fatty acid radical to form an organic peroxide. The peroxide is unstable

and decomposes to form more organic free radicals, which in turn form more organic peroxides (Recknagel and Glende, 1973). This process appears to lead to fatty acid chain decomposition, with the resulting breakdown of membrane structure (Recknagel and Glende, 1973). This breakdown may lead to a halt in lipid excretion via the Golgi apparatus, with fatty liver occurring as a consequence. Cell necrosis would also follow directly from lipid destruction. The mechanism by which lipid peroxidation could lead to cell transformation is not explained at present, and the molecular events leading to CCl_4 carcinogenicity remain unknown.

In addition to these proposed mechanisms of toxicity, two minor metabolic pathways have been postulated: dimerization of two trichloromethyl free radicals to form hexachloroethane (Fowler et al., 1969) and the formation of a trichloromethyl peroxy radical which may result in production of phosgene and carbon dioxide (Shah et al., 1979). Both hexachloroethane and phosgene are toxic, but the extent of their contribution to observed hepatotoxicity is unknown. Carbon tetrachloride has not been found to bind to cellular polynucleotides, but presently only one investigation studying the binding of CCl_4 to such nucleotides has been reported.

9. TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS

9.1. TERATOGENICITY

In two inhalation studies on the teratogenic and prenatal toxicologic effects of carbon tetrachloride, the chemical was reported to produce prenatal toxicity but not teratogenicity. In the first to be discussed, Schwetz et al. (1974) exposed pregnant Sprague-Dawley rats to carbon tetrachloride at 1800 or 6300 mg/m^3 for 7 hours/day on days 6 to 15 of gestation. Statistically significant decreases in fetal body weight and crown-rump length were observed. Other parameters examined such as sex ratio, live fetuses/litter and resorptions were not significantly different from those of controls. Two other statistically significant fetal effects were noted: an increased incidence of litters with sternebral anomalies in the 6300 mg/m^3 group and an increased incidence of litters with subcutaneous edemas in the 1800 mg/m^3 group. The incidence of litters with edema in the 6300 mg/m^3 group (50%), although apparently increased, was not significantly different from the control incidence (33%). The dams exposed to both concentrations of carbon tetrachloride showed a decreased food consumption compared to control animals and a statistically significant decrease in weight gain. Both of these effects were greater at the higher dose level. Hepatotoxicity, as measured by significantly increased SGPT activity, was also seen in the dams following daily exposure to 1800 or 6300 mg/m^3 CCl_4 but the increase was greater at the lower dose level. The authors did not establish any consistent pattern between fetal toxicity and maternal toxicity at the subanesthetic levels of carbon tetrachloride used in this experiment. They concluded that carbon tetrachloride was not highly embryotoxic at the concentrations used in this experiment. The evidence of maternal toxicity precluded any statement about the teratogenic potential of CCl_4.

Another study reported no teratogenic effects following exposure of pregnant rats to carbon tetrachloride at 1575 mg/m^3 8 hours/day for five consecutive days between days 10 to 15 of pregnancy (Gilman, 1971). Concomitant exposure to 15% ethanol in drinking water also did not result in teratogenic effects. Carbon tetrachloride exposure, however, did decrease the viability index to 83% as compared to 99% for controls ($p<0.1$), resulting in a decrease in the number of pups per litter, 9.2 as compared to 10.3 for controls. The lactation index was also decreased to 83% as compared to 98% for controls. Concomitant ethanol exposure exacerbated the former effect: 8.48 pups per litter as compared to 10.3 for controls. Although the number of animals used was small, 10 control and 25 experimental, thus lessening the sensitivity, the results of this study tend to support those of Schwetz et al. (1974), indicating prenatal toxicity.

Subcutaneous exposure of carbon tetrachloride to pregnant rats has been reported to result in liver damage in fetuses and neonates (Bhattacharyya, 1965). Administration of 1600 mg/kg CCl_4 subcutaneously on days 20 or 19 of gestation resulted in small areas of focal hepatic necrosis in neonates born 48 or 72 hours later, respectively. Histologic findings generally included a sharply demarcated area of centrilobular necrosis and proliferation changes in nonnecrotic lobes.

These authors also treated fetuses directly with carbon tetrachloride by subjecting the mother to a laparotomy and either injecting the chemical directly into the fetus or into the amniotic sac through the uterine wall (Bhattacharyya, 1965). Liver changes following injection of 6 mg carbon tetrachloride were variable; cells generally became extremely pale in centrilobular and midzonal areas, indicating fatty infiltration. Livers remained abnormal until at least 4 days after birth. No necrosis, hemorrhage or regeneration was observed.

Sensitivity of the liver of neonate rats to carbon tetrachloride was reported to be low 1 hour after birth, then to rise above the adult level at 19 hours and to decline to adult levels by 3 to 7 days after birth. Thus, only 2 of 10 1-hour-old neonates receiving carbon tetrachloride (1600 mg/kg) subcutaneously showed centrilobular necrosis after 24 hours. In addition, hepatic portal areas contained numerous neutrophils, but in contrast to findings in adult animals, no bile duct proliferation could be observed. Nineteen-hour neonates showed more pronounced hepatic damage than 1-hour neonates. Damage declined in 3- and 4-day-old neonates; that in 5-, 6- and 7-day-olds was similar in appearance to that of adults.

Neonates can apparently be exposed to CCl_4 through mothers' milk (Bhattacharyya, 1965). Subcutaneous administration of carbon tetrachloride at 1600 or 3200 mg/kg bw to four nursing rats resulted in hepatic damage in the neonates 24 or 48 hours later. A dose of 800 mg/kg bw to dams did not produce any hepatic damage to offspring. Levels in the milk were not reported.

The pre- and postnatal toxicologic studies described above do not meet current design criteria for hazard assessment purposes in that fewer than three dose levels were used and a second, nonrodent species was not studied (U.S. EPA, 1981). Furthermore, positive controls were not used. No study of specific postnatal functional lesions was available.

9.2. OTHER REPRODUCTIVE EFFECTS

Testicular degeneration was observed in rats receiving carbon tetrachloride at 4800 mg/kg bw i.p. (Chatterjee, 1966). One group of six male rats received carbon tetrachloride as a 1:1 mixture in coconut oil. The vehicle control group received only an equal volume of coconut oil. On day 15 all animals were sacrificed. Body weights were similar for treated and

control animals. However, the relative testes weight decreased from 15.5 (±0.4) g/kg bw in controls to 9.8 (±1.2) g/kg bw in exposed animals. A decrease in testis size and weight is a good indicator of a decline in male spermatogenic process. Relative weight of seminal vesicles showed an even more pronounced decrease: 1.27 (±0.171) g/kg bw in treated as compared to 3.10 (±0.059) g/kg bw in control animals. Relative pituitary weight was, however, increased: 50.0 (±1.4) mg/kg bw in treated as compared to 32.4 (±0.9) mg/kg bw in control animals. This increase would be expected if feedback mechanism from the testis is obliterated and pituitary concentrations of gonadotrophin were increased. However, in this study, actual pituitary content of gonadotrophins was not measured. Therefore, no conclusions can be made on the effect of the chemical on the feedback mechanism. Thus, the significance of the increase in pituitary weights is difficult to interpret.

Histological examination of testes in CCl_4-treated animals showed testicular atrophy and "some abnormality" in spermatogenesis. The authors proposed a mechanism for carbon tetrachloride-induced testicular atrophy in which blockage of pituitary hormone release results in atrophy of Leydig cells within the seminal vesicles, followed by an abnormal spermatogenesis.

In another study (Kalla and Bansal, 1975), intraperitoneal administration of carbon tetrachloride (4800 mg/kg bw as a 1:1 mixture of coconut oil) to male rats for 10, 15 or 20 days (Group I, II or III, respectively) led to impairments in spermatogenesis as indicated by histological examination. Vehicle controls were administered equal volumes of coconut oil. Weights of testes, seminal vesicles, epididymis and prostates were decreased in exposed animals, whereas the weight of adrenals increased (Table 9-1). The gonadosomatic index (GSI) (equal to body weight x testes weight/100) was also

TABLE 9-1

Weight Changes in Male Rat Reproductive Organs After Carbon Tetrachloride Treatment[a,b]
(4800 mg/kg bw i.p. as a 1:1 mixture of coconut oil)

Treatment and Period	Body Weight (g)		Testis (g/kg bw)	Seminal Vesicles (g/kg bw)	Epididymis (g/kg bw)	Prostate (g/kg bw)	Adrenal (g/kg bw)	GSI[c]
	Before Treatment	After Treatment						
Group I 10 days								
Control	257± 5.0	269± 4.5	12.36±0.94	4.25±0.55	4.45±0.45	2.1 ±0.7	0.1d±0.02	7.94±0.45
Treated	257±13.71	247±10.76	8.84±1.04	2.41±0.14	2.88±0.38	1.26±0.24	0.24±0.03	5.68±0.31
Group II 15 days								
Control	230±15.49	235± 5.0	10.47±0.59	3.9±0.52	4.12±0.18	2.19±0.31	0.22±0.01	6.02±0.21
Treated	230±15.49	173±13.42	9.46±0.33	1.17±0.26	2.63±0.18	1.68±0.13	0.32±0.05	5.44±0.28
Group III 20 days								
Control	234± 5.5	235± 6.5	11.37±0.06	3.24±0.31	3.72±0.09	1.78±0.04	0.12±0.01	6.64±0.23
Treated	230± 3.5	219± 0.5	9.9 ±0.65	1.53±0.28	2.52±0.22	1.29±0.01	0.25±0.02	5.69±0.38

[a]Source: Adapted from Kalla and Bansal, 1975

[b]Mean ± standard deviation

[c]Gonadosomatic index = body weight x testis weight/100

decreased in treated animals. A slight decrease in pituitary weight was observed following a 10-day treatment but not after either the 15- or 20-day treatment. As reported, the ratio of germinal to nongerminal area steadily decreased from Group I to Group III and was always higher in treated than control animals. Significant differences in total germinal area between treated and control animals, however, were observed only at 20 days. Histological examination did not reveal any abnormalities in testes from Group I. Clusters of mature sperm were present in the lumen. In Group II, slight testicular damage was observed: a decrease in spermatogenic cells and increased lumen size. In Group III, shrinkage of the tubules and increased area of the lumen were observed. Arrangement of the germ cells was disrupted; early gonadal cells were present in the lumen of many of the tubules. No spermatids were observed. Interstitial material was "damaged" and in many places the basement membrane was detached from the epithelium.

Problems associated with this study include that the authors do not report numbers of animals studied at each dose level, frequency of treatments, or whether the dose of 3 mℓ/kg bw was the total dose or the dose given at each treatment, although the former is assumed. Thus, noting the ambiguity in the reporting of dose levels, CCl_4 at an assumed total dose of 3 mℓ/kg bw over 10 days had a distinct but minor effect on male rat reproductive physiology, whereas a total dose of 96 g/kg bw over 20 days resulted in severe disturbances of spermatogenesis. In view of the increased pituitary weight and the decreased adrenal weight, a possible mechanism of action of carbon tetrachloride's male reproductive effect could be the suppression of pituitary gonadal axes. However, as pointed out earlier in this section, pituitary weight by itself is almost a meaningless parameter. Many hormones are located in the total pituitary, and reproduc-

tive hormones, such as gonadotrophins, are located only in the anterior pituitary. In addition, before a conclusion can be made about pituitary content of various hormones, measurements of the synthesized, stored and released hormones should be made. Despite these criticisms, the two studies described in this section had shortcomings with regard to current experimental design criteria for hazard assessment: less than three dose levels and no data for a second, nonrodent species.

Teratogenic effects in humans caused by carbon tetrachloride exposure have not been reported. However, human fetuses in one study appeared to have selectively accumulated carbon tetrachloride from the mothers' circulation (Dowty and Laseter, 1976). Maternal blood samples were taken from 11 women either before or directly after (vaginal) delivery (prior exposure of the women to toxic chemicals was not reported). Paired cord blood samples were obtained immediately after delivery. All volatiles were analyzed by gas chromatography and mass spectrometry. Carbon tetrachloride, benzene and chloroform were present in higher concentrations in cord blood as compared to maternal blood.

9.3. SUMMARY

Carbon tetrachloride has produced prenatal toxic effects, some of which (i.e., subcutaneous edema) could not be associated with extent of maternal exposure. Rats exposed to carbon tetrachloride *in utero* have shown hepatic abnormalities at birth, but the fetal rat liver appears to be less sensitive than the adult liver to the hepatotoxic effects of CCl_4.

Carbon tetrachloride has produced distinct degenerative changes in testicular histology, eventually resulting in aspermatogenesis and functional male infertility. These effects occurred following intraperitoneal injection at relatively high doses. Unfortunately, low doses were not tested.

Due to the limited number and scope of the studies reported in this chapter, it is difficult to evaluate the potential of CCl_4 to cause adverse teratogenic, embryotoxic or reproductive effects. Some of the specific limitations are provided within the discussion of each study. In general, the studies do not provide adequate dose groups for concluding the existence of teratogenic or reproductive effects (according to testing criteria such as those currently used for U.S. EPA Office of Pesticides Programs or Office of Toxic Substances).

10. MUTAGENICITY

10.1. RELEVANT STUDIES

Studies to determine the mutagenic activity of carbon tetrachloride in the Salmonella typhimurium revertant system have been uniformly negative. A review article written by McCann et al. (1975) stated that an assay using Arochlor-induced S9 activation and strains TA100 and TA1535 was negative. The McCann et al. article contained no details of the procedure used to generate this negative result. Another review article (Fishbein, 1976) in which no data were presented, contained a statement that carbon tetrachloride was not mutagenic when assayed in a spot test with the TA1950 strain. Simmon and Tardiff (1978) assayed the mutagenicity of carbon tetrachloride as a gas in a desiccator to avoid loss of the substance to the atmosphere. It was previously found that many volatile alkyl halides are mutagenic only when tested in a desiccator apparatus. Negative results were obtained in this study with strains TA1535 and TA100 with and without Arochlor-induced rat liver S9 activation.

In an abstract, Uehleke et al. (1976) reported that carbon tetrachloride was not mutagenic in Salmonella typhimurium (strains TA1535 and TA1538) and Escherichia coli K12 incubated with rabbit liver microsomes. However, these results cannot be evaluated because no data were presented. Uehleke et al. (1977) studied the interaction of carbon tetrachloride with liver microsomes from phenobarbital-treated rabbits and used this system for suspension assays with Salmonella typhimurium strains TA1535 and TA1538. About 10% of the ^{14}C-labeled carbon tetrachloride (1mM) was covalently bound to endoplasmic protein and greater than 30% was bound to microsomal lipid. (The metabolism of carbon tetrachloride with subsequent lipid peroxidation is presumably one mechanism for the liver toxicity observed in animals and humans exposed to carbon tetrachloride. See Section 8.3.) Interaction of

carbon tetrachloride with nucleic acid was not tested. No mutagenic activity was observed in the bacteria, which were incubated under nitrogen gas in tightly closed test tubes with 8 mM (1.23 g/ℓ) carbon tetrachloride and microsomes. Since the solubility of carbon tetrachloride in water is 0.8 g/ℓ at 25°C, 1.23 g/ℓ may be just above the solubility level at 37°C. The authors concluded that a reactive species (such as free radicals) generated in the biological system may not distribute into the incubation medium and, thus, may be inaccessible to the test bacteria. They also speculated that any potential reactive metabolites of carbon tetrachloride may be very short lived.

Callen et al. (1980) carried out a study in yeast that was designed to overcome the problem of a short-lived intermediate's having to react with inacessible DNA. The D7 strain of Saccharomyces cerevisiae contains an endogenous cytochrome P-450 dependent mono-oxygenase activation system. Three different genetic effects can be examined under this system: gene conversion, mitotic recombination and gene reversion. These effects were measured by using cells exposed in suspension at 3.23, 4.31 and 5.13 g of carbon tetrachloride per liter of buffer, well above the solubility level of carbon tetrachloride in water (0.8 g/ℓ at 25°C). Therefore, a dose-response relationship could not be obtained because the dose was essentially the same in all cases - the solubility level of carbon tetrachloride in water at 37°C. Volatilization of the carbon tetrachloride is not expected to have occurred to any significant extent, because the incubations were carried out in screw-capped glass tubes. Although the dose is essentially constant, amounts in suspension will vary. Extracellular or membrane effects may result in the high toxicity observed at 5.13 g/ℓ. Results of the Callen et al. study are presented in Table 10-1. A 1-hr treatment of

TABLE 10-1

Mutagenic Effects of Carbon Tetrachloride following 1-hour treatment at 37°C on Strain D7 of *Saccharomyces cerevisiae*[a,b]

	Concentration (mg/ℓ)			
	0	3234	4312	5128
Survival				
Total colonies	1454	1252	1120	152
% of control	100	86	77	10
trp-5 locus (gene conversion)				
Total convertants	285	331	350	506
Convertants/10^5 survivors	2.0	2.6	3.1	61.7
ade-2 locus (mitotic recombination)				
Total twin spots	1	3	3	10
Mitotic recombinants/10^4 survivors	1.6	5.3	5.8	40.1
Total genetically altered colonies	11	19	16	65
Total genetically altered colonies/10^3 survivors	1.7	3.4	3.1	33.3
ilv-1 locus (gene reversion)				
Total revertants	38	41	57	11
Revertants/10^6 survivors	2.6	3.3	5.1	7.2

[a]Source: Adapted from Callen et al., 1980

[b]The total number of colonies in the different classes represent total counts of colonies from five plates in the case of survival, conversion and revertant-frequency estimations. Mitotic recombination was estimated from counts of colonies growing on a total of 30 plates, 20 plates containing medium of which all surviving cells grew and 10 plates containing medium on which only trp-5 convertants grew.

cells with carbon tetrachloride at the highest amount treated resulted in significant increases in gene conversion and mitotic recombination. However, survival was only 10% at this dose. Therefore, increases in gene conversion and mitotic recombination may be influenced by the high toxic levels used. Alternatively, since concentrations in these studies were very high (above the solubility), the increases in these frequencies may be caused by an impurity in the carbon tetrachloride sample used in this study. Information on the identification and concentration of impurities is forthcoming from Mallinckrodt.

There was questionable increase in gene reversion. The greatest value of total revertants counted with carbon tetrachloride-treated cells (57 at 4.31 g/ℓ) was less than the control value (61) in the chloroform experiment reported in the same study. The authors did not demonstrate that the increase in revertant frequency observed represented true mutation induction and not simply selective killing.

Negative results have also been obtained in a recently developed in vitro chromosome assay that utilized an epithelial-type cell line derived from rat liver (Dean and Hudson-Walker, 1979). This cell line has sufficient metabolizing activity to activate various chemical mutagens and carcinogens without the need for an extrinsic activating system. Sealed-flask cultures were treated with carbon tetrachloride dissolved in growth medium at 0.005, 0.010 and 0.020 mg/ℓ. Carbon tetrachloride did not induce any chromosomal aberrations, whereas a number of direct-acting mutagens and several requiring metabolic activation produced chromatid breaks, gaps and exchanges.

Rocchi et al. (1973) studied the binding of carbon tetrachloride with nucleic acids and protein. ^{14}C-Labeled carbon tetrachloride (367 µmol/kg) was injected into rats and mice following which the amount of

metabolite(s) of carbon tetrachloride that covalently bind to liver DNA, RNA, nuclear proteins and cytoplamic proteins was measured. The authors reported that a significant amount of labeled material was found to be associated with RNA, nuclear proteins and cytoplasmic proteins in rats. Rats pretreated with 3-methylcholanthrene (5 mg, 24 hr before treatment with carbon tetrachloride) increased the amount of label associated with the macromolecules. No label was associated with DNA in the rat studies. Similar studies in mice indicated that DNA was labeled but only after pretreatment with 3-methylcholanthrene (1 mg, 24 hr before carbon tetrachloride dosing).

In an _in vitro_ experiment, Rocchi et al. (1973) used rat or mouse liver microsomes to activate labeled carbon tetrachloride in the presence of calf thymus DNA. They found that pretreatment of animals with 3-methylcholanthrene enhanced the amount of label associated with DNA. Furthermore, pH 5 enzyme preparations were also found to increase the amount of label bound to DNA. Therefore, from these results, it appears that carbon tetrachloride (metabolites) can interact with DNA, but that for optimal binding conditions, microsomal enzymes had to be activated with 3-methylcholanthrene in the presence of pH 5 enzyme preparations.

10.2. SUMMARY

Carbon tetrachloride has been tested for its mutagenic potential in bacteria, yeast and a mammalian cell line. All point mutation studies were negative. It is conceivable that potentially mutagenic reactive intermediates of carbon tetrachloride (such as the free radical $\cdot CCl_3$) are generated in an S9 system but that they are too short-lived to interact with DNA in _in vitro_ test systems. The Callen et al. study (1980) was designed to overcome this problem by the use of an _in vivo_ activation system in

yeast. However, because of other problems with this study and the lack of corroborative studies, the evidence is inadequate to conclude that carbon tetrachloride is not genotoxic.

Binding studies by Rocchi et al. (1973) indicate that carbon tetrachloride can interact with DNA but in order to achieve optimal conditions, certain precautions must be taken. Therefore, some of the negative point mutation test results that have been reported may be due to inadequate activation of carbon tetrachloride to a metabolite capable of causing mutations. Additional tests should be conducted at levels for which the toxicity of carbon tetrachloride is not a factor and appropriate measures should be taken to assure that activation is occurring.

11. CARCINOGENICITY

The carcinogenic effects of carbon tetrachloride have been well documented. The International Agency for Research on Cancer (IARC) concludes that the evidence from animal studies demonstrating CCl_4-induced hepatic neoplasms is sufficient to indicate experimental animal carcinogenesis (IARC, 1979). The National Cancer Institute (NCI) also identifies CCl_4 as an animal carcinogen and has used it as the positive control in three of its bioassays.

This section will focus on the carcinogenicity of CCl_4 demonstrated in various species. Some of the studies showing carcinogenesis have also shown severe toxic effects in the same animals. A discussion of these toxic effects is included here to offer a clearer picture of the induced malady.

11.1. RATS

Studies performed on rats have primarily used subcutaneous injection as the route of exposure. Hepatomas were found as well as toxic effects such as cirrhosis, hyperplasia and cholangiofibrosis. Neoplasms also developed following exposure by oral ingestion.

Cameron and Karunaratne (1936) looked at CCl_4 cirrhosis in relation to liver regeneration in the rat. Albino rats weighing about 150 g each were administered subcutaneous injections of 0.1 to 0.25 mℓ carbon tetrachloride twice a week. After 6 to 10 doses, changes which developed in the liver disappeared within 7 to 10 days after cessation of treatment. With longer periods of exposure, the liver showed less and less tendency to return to a normal appearance when the chemical was discontinued. Cirrhosis of the liver developed after several doses and was severe and irreversible after 40 doses.

The liver was pale, tough and finely granular. There was extensive fibrosis radiating from the portal areas, thereby dividing the liver into

small irregular masses. Hyperplastic nodules were seen in different parts of the liver.

In this study, rats given subcutaneous injections of carbon tetrachloride readily developed cirrhosis of the liver. Also, there were hyperplastic nodules of the liver.

Reuber and Glover (1967) administered subcutaneous injections of CCl_4 twice a week for 12 weeks to inbred Buffalo male and female rats 4, 12, 24 and 52 weeks old. There were 10 to 14 rats of each sex and age. Additionally, newborn rats were obtained and given CCl_4 at 4 days of age. All rats were given 1.3 mℓ/kg bw of a 50% solution of CCl_4 and corn oil. Control rats, six per group, were injected with the same amount of corn oil.

The 4-day-old animals died in an average of 8 days with hepatic and renal necrosis. The other rats survived for the 12 weeks of the study. During this period, the 52-week-old rats maintained their weight, and the 12-week-old rats each gained from 20 to 30 g. The 4-week-old females weighed three times their starting weights and males weighed four times their starting weights.

At sacrifice complete necropsies were done. All organs were examined histologically, including such tissues as diaphragm, tongue and skeletal muscle. Special staining was done for glycogen, mucin, connective tissue, ceroid, canaliculi, hemosiderin and lipid.

The males given subcutaneous injections at 52 weeks of age had more hyperplastic lesions than the other males. Six of 14 rats (43%) had hyperplastic nodules, with one having a small hepatic carcinoma. The only other males with nodules were the 24-week-old rats, 2/11 (18%). The remaining 52-week-old rats, and all but one of the 24-week-old rats, had hyperplasia of the liver. Hyperplasia developed in less than half of the 12-week-old rats. Hyperplastic lesions and hyperplasia were not observed in control male rats.

The 24- and 52-week-old females had more hyperplastic nodules than did the younger females. The most striking lesions were in the 24-week-old rats. In this group, 8/10 rats (80%) had hyperplastic nodules and one rat had a small carcinoma of the liver. There were more hyperplastic nodules per liver and larger lesions in the females than in the males. Lesions were not present in control female rats.

There were two kinds of hyperplastic lesions in the liver, one located in the periportal region and the other around central veins. Cirrhosis varied from mild to severe, but was unrelated to the hyperplastic lesions in indivicual rats. The severity and the histologic pattern of the cirrhosis were related to age and sex. The hyperplastic nodules seen were similar to those known to be preneoplastic (Reuber and Glover, 1967). If the study had been continued for a longer period of time, it is possible that the hyperplastic nodules could have become overt tumors. Results of this study are given in Table 11-1.

In summary, 24- and 52-week-old rats of both sexes given subcutaneous carbon tetrachloride developed more hyperplastic hepatic nodules, as well as an occasional early carcinoma of the liver, than did rats of other ages. The number of hyperplastic lesions per liver and the size of lesions were larger in females than in males. Four-day-old rats died with necrosis of the liver and kidney.

Reuber and Glover (1967) also studied cholangiofibrosis of the liver in male and female Buffalo strain rats of varying ages. Cholangiofibrosis, may be a precursor of cholangiocarcinomas of the liver; it is a lesion composed of ducts lined by irregular epithelial cells and surrounded by connective tissue (Reuber and Glover, 1967). Cholangiofibrosis of the liver developed in male and female rats receiving injections of carbon tetrachloride. The lesion was present in male rats of all ages, except those 4 weeks of age.

TABLE 11-1

Lesions of the Liver in Rats Given Subcutaneous Carbon Tetrachloride*
(1.3 mℓ/kg bw in 50% solution with corn oil)

Age (weeks)	Hyperplasia	Hyperplastic Nodules	Carcinoma	Total Nodules Plus Carcinoma
MALE				
4	6/14 (43%)	0/14 (0%)	0/14 (0%)	0/14 (0%)
12	4/11 (36%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
24	8/11 (73%)	2/11 (18%)	0/11 (0%)	2/11 (18%)
52	7/14 (50%)	6/14 (43%)	1/14 (7%)	7/14 (50%)
FEMALE				
4	4/11 (36%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
12	5/11 (45%)	3/11 (27%)	0/11 (0%)	3/11 (27%)
24	1/10 (10%)	8/10 (80%)	1/10 (10%)	9/10 (90%)
52	4/11 (36%)	6/11 (54%)	1/11 (9%)	7/11 (64%)

*Source: Reuber and Glover, 1967

The lesion was increased in male rats 5 weeks of age given both CCl_4 and 3-methylcholanthrene (MCA), whereas it was decreased in rats of all other ages. Most female rats given both chemicals also had cholangiofibrosis.

The comparative carcinogenicity of carbon tetrachloride has been studied in five rat species: Japanese, Osborne-Mendel, Wistar, Black and Sprague-Dawley (Reuber and Glover, 1970). Groups of 12 to 17 male rats of each strain were given twice weekly subcutaneous injections of carbon tetrachloride (2080 mg/kg ow as a 50% solution in corn oil). Treated animals were killed when moribund; controls for each strain were killed at the same time as the last experimental animal. Incidence of hepatic lesions is given in Table II-2. Lesions other than hepatic also occurred. Hemangiomas of the spleen were present in two Japanese rats and in one of the Osborne-Mendel strain. There were carcinomas of the thyroid gland in three Osborne-Mendel and three Japanese rats. One Japanese rat had a subcutaneous leiomyosarcoma; two Osborne-Mendel and three Japanese rats had chronic renal disease. The data indicate that: (1) sensitivity to carbon tetrachloride-induced neoplasms varies widely among strains; and (2) the trends in incidence of neoplasms and cirrhosis appear to be inversely related. Varying amounts of toxicity occurred: all experimental animals of the Black rat strain were dead at 18 weeks, and those of the Sprague-Dawley strain at 16 weeks; the failure to find carcinomas in those strains may have been caused in part by an insufficient latency time. In all three other strains, toxicity (e.g., cirrhosis, hepatic vein thrombosis, cholangiofibrosis) occurred. Toxicity (and survival time) were inversely related to carcinogenicity. It thus appears that there is no causal connection between the degree of toxicity and carcinogenicity.

TABLE 11-2

Evidence of the Most Advanced Lesions in Rats Administered Carbon Tetrachloride* (2080 mg/kg bw in 50% solution with corn oil)

Lesion	Japanese	Osborne-Mendel	Wistar	Black	Sprague-Dawley
No hyperplasia	0/15	0/13	0/12	4/17	8/16
Hyperplasia	0/15	1/13	1/12	6/17	6/16
Hyperplastic nodule	3/15	4/13	7/12	7/17	2/16
Small carcinoma	4/15	4/13	3/12	0/17	0/16
Large carcinoma	8/15	4/13	1/12	0/17	0/16
Total carcinoma	12/15	8/13	4/12	0/17	0/16
No cirrnosis	0/15	0/13	0/12	0/17	0/16
Mild cirrnosis	9/15	2/13	0/12	0/17	0/16
Moderate cirrhosis	5/15	7/13	6/12	4/17	0/16
Severe cirrhosis	1/15	4/13	6/12	13/17	16/16

*Source: Adapted from Reuber and Glover, 1970

In an NCI (1976) bioassay for trichloroethylene, carbon tetrachloride was used as the positive control. The positive control groups of 50 Osborne-Mendel rats of each sex were administered CCl_4 in corn oil by gavage five times weekly for 78 weeks at two dose levels: 47 and 94 mg/kg bw for males, 80 and 159 mg/kg bw for females. This treatment resulted in some toxicity (cirrhosis, fatty liver) and death: at 110 weeks at the high dose, only 7 of 50 males and 14 of 50 females survived and at the low dose, 14 of 50 males and 26 of 50 females survived as compared to 26 of 100 males and 51 of 100 females for controls. The median survival times were 92 and 102.5 weeks for males given the low and high doses, respectively and 67.5 and 102.5 for females given the low and high doses, respectively. The incidence of hepatocellular carcinomas was increased in animals exposed to CCl_4 as compared to pooled colony controls (Table 11-3). However, this was statistically significant only for low dose females as compared to the colony controls and not the matched controls. Absolute incidence of hepatic neoplasms was low (≈5% in the animals exposed to CCl_4). This may be attributed to the resistance by this rat strain to such chlorinated hydrocarbons. The apparent decrease in the incidence of hepatocellular carcinomas in female rats at the high dose was attributed to increased lethality (i.e., females died before tumors could be expressed). The incidence of other neoplasms was acknowledged but not quantified. This study is used by the National Research Council in determining the carcinogenic risk estimate for CCl_4 due to the dose levels used and the appropriate length of the study (NAS, 1978).

11.2. MICE

Several studies have been reported which indicate induction of liver tumors in various strains of mice treated with CCl_4 either by oral inges-

TABLE 11-3

Incidence of Liver Tumors in Carbon
Tetrachloride-Treated Rats and Colony Controls*

	Animal Group		Hepatocellular Carcinoma	Neoplastic Nodule
Males	Controls		1/99	0/99
	Low dose	(47 mg/kg bw)	2/50	2/50
	High dose	(90 mg/kg bw)	2/50	1/50
Females	Controls		0/98	2/98
	Low dose	(80 mg/kg bw)	4/49	2/49
	High dose	(159 mg/kg bw)	1/49	3/49

*Source: NCI, 1976

tion which has been the primary route of exposure, subcutaneous injection or rectal administration. Signs of liver toxicity such as necrosis and cirrhosis have also been a frequent result of CCl_4 treatment in the carcinogenicity studies in mice.

In a study by Andervont (1958), groups of 30 to 77 female or male C3H mice were administered 6.46 mg CCl_4 by gavage once weekly for 2 weeks, followed by administration of 9.6 mg CCl_4 once weekly for 17 weeks (equivalent of 213 and 320 mg/kg bw). Pathogen-free C3H mice were used. For males, no difference in the incidence of hepatomas was observed between the pathogen-free and pathogen-carrying groups combined, and control mice: 79% as compared to 49%. The average number of hepatomas per animal was 1.8 in treated animals (pathogen-free and pathogen-carrying combined) and 1.3 in controls. In females, a difference between the incidence of hepatomas in pathogen-free and pathogen-carrying mice was observed: 46 and 29%, respectively, as compared to 3% in controls. The average number of hepatomas per female mouse in the pathogen-free, pathogen-carrying and control groups was 1.5, 1.2 and 1.0, respectively, indicating that both the incidence as well as the average number of tumors per animal increased in the order: control mice < treated normal mice < treated pathogen-carrying mice.

A study by Edwards (1941) also reported on the induction of hepatomas in mice by exposure to carbon tetrachloride. Two hundred and seven male C3H mice, aged 3 to 6 months, and 133 male and female strain A mice, aged 2 to 3.5 months, were used. They were given 0.1 mℓ of a 40% olive oil solution of carbon tetrachloride (0.04 cc CCl_4) by stomach tube two or three times weekly for 8 to 16 weeks. Autopsy was performed up to 21 weeks after the last treatment.

Olive oil was administered by stomach tube in doses of 0.1 mℓ 2 or 3 times weekly to control male C3H and A strain mice from the same stock as those mice used in treated groups. Twenty-three strain C3H mice were given CCl_4 from 39 to 50 times and were killed and examined from 9 to 11 months of age. A high percentage of the treated animals developed hepatomas.

Of 143 C3H mice, which varied from 6 to 10 months of age at autopsy, 126 (88.1%) showed hepatomas (Table 11-4). Similar tumors were present in all of the 54 strain A mice whose ages varied from 4.5 to 12 months (Table 11-5). It should be noted that the incidence of spontaneous hepatomas in both the C3H and A strains is markedly below that of the induced tumors in the treated mice. Autopsies performed on 17 C3H male mice 8.5 to 9 months of age and the same stock as that used in the study failed to show any hepatic tumors.

In 1942, Edwards et al. performed another study on mice. The mice used in this study were inbred strain L (their incidence of spontaneous hepatomas is extremely low), 2.5 to 3.5 months or 3.5 to 7.5 months of age at the start. The number of mice varied from 8 to 39 per group. Carbon tetrachloride of a high degree of purity was administered in olive oil by stomach tube usually three, but occasionally two, times weekly. Each treatment consisted of 0.1 cc of a 40% solution or 0.04 mℓ of CCl_4. Mice were given 46 administrations of CCl_4 over a 4-month period and were killed and necropsied 3 to 3.5 months after the last treatment. The mice varied from 8.5 to 14 months of age at necropsy. The liver was examined histologically.

Thirty-four of 73 mice (47%) given CCl_4 developed hepatomas. The incidence of tumors in the younger mice was essentially similar to that for the older mice, with the exception of the older females where the incidence was considerably lower. Tumors of the liver were observed in 7/15 younger

TABLE 11-4

Incidence of Tumors in C3H Mice Ingesting Carbon Tetrachloride*

Group	Number of Mice Autopsied	Number of Mice with Hepatomas	Incidence of Hepatomas (in percent)
Controls	17	0	0
Controls w/Olive oil	23	1	4.3
Treated Animals (Olive oil and 0.04 mℓ CCl_4)	143	126	88.1

*Source: Edwards, 1941

TABLE 11-5

Incidence of Tumors in Strain A Mice Ingesting Carbon Tetrachloride*

Group	Number of Mice Autopsied	Number of Mice with Hepatomas	Incidence of Hepatomas (in percent)
Controls	200	1	0.5
Controls w/Olive oil (0.1 mℓ 2 or 3x weekly)	22	0	0
Treated Animals (0.01 mℓ of a 40% soln. of CCl_4 in Olive oil 2 or 3x weekly)	54	54	100.0

*Source: Edwards, 1941

male mice (47%), 21/39 older male mice (54%), 3/8 younger females (38%), and 3/11 older females (27%). Cirrhosis of the liver was not mentioned.

Hepatomas were observed in 2/152 (1%) untreated strain L mice. One of 23 untreated virgin male mice (4%) and 0 of 28 females (0%), necropsied at 15 months of age, had tumors of the liver. Tumors were not present in 22 males and 28 females 18 months of age or in 27 female breeders 12 to 23 months of age. One of 24 male breeders (4%) had a tumor. The results are summarized in Table 11-6.

In summary, strain L male and female mice were highly susceptible to the induction of hepatomas by carbon tetrachloride, and male mice were slightly more susceptible than female mice.

Eschenbrenner and Miller (1943) studied the effects of size and spacing of multiple CCl_4 doses in the induction of hepatomas. Strain A mice were used because of their normal low incidence of tumors of the liver in untreated mice ($\leq$1%). Male and female mice were 2.5 to 3 months old at the beginning of the study.

Solutions of CCl_4 in olive oil were administered by stomach tube. All mice received 30 doses of the solution or olive oil alone. Five dilutions of CCl_4 were used: 32, 16, 8, 4 and 2% solutions. Mice received 0.005 mℓ of solutions per gram bw containing 16×10^{-4}, 8×10^{-4}, 4×10^{-4}, 2×10^{-4} or 1×10^{-4} mℓ, respectively, of CCl_4. Central necrosis of the liver was produced by each of these doses. Control mice received 0.005 mℓ olive oil/g bw.

The experimental and control groups were divided into five subgroups according to the interval between successive doses (1, 2, 3, 4 and 5 days) and the total period of treatment (29, 58, 87, 116 or 145 days). Equal numbers of male and female mice were used in each of the experimental and the

TABLE 11-6

Tumors of the Liver in Male and Female Mice Receiving Carbon Tetrachloride by Stomach Tube[a] (0.04 mℓ CCl_4 2 or 3x weekly)

Age (months)	Males	Females
2.5 - 3.5	7/15 (47%)	3/8 (38%)
3.5 - 7.5	21/39 (54%)	3/11 (27%)
2.5 - 7.5[b]	28/54 (52%)[c]	6/19 (32%)[c]

[a]Source: Edwards et al., 1942

[b]These values represent total number of tumors observed in mice in both age groups.

[c]Old control mice of this strain exhibit a very low incidence, as compared to CCl_4-treated mice. Hepatomas were present in 2/71 untreated males (3%) and 0/81 untreated females (0%).

five control groups. All mice were examined for the presence of hepatoma 150 days after the first dose. Some of them were killed at that time; others were subjected to laparotomy. If hepatomas were not present, laparotomies were performed at monthly intervals thereafter to determine if hepatomas eventually did appear. The gross diagnoses of hepatoma were confirmed by histological examinations.

In the lower dosage and shorter interval groups, hepatomas were few in number and small in size. With increases in dose and in intervals between successive doses, there was progressive increase in the number of small hepatomas and the size of hepatomas for a given mouse. There was no difference in the incidence of tumors of the liver between males and females.

The authors present the data in a matrix showing dose by interval. An adaptation of their matrix is given in Table 11-7. Excluding the values for the 1-day interval, the data were summed across intervals and sexes. A χ^2 test for trend among proportions was significant at $p < 0.01$.

In this study, the incidence of hepatomas roughly increased non-linearly with the total duration of CCl_4 exposure. A given incidence of hepatomas was obtained with progressively less total amount of carbon tetrachloride as the duration of administration was increased.

In another study, liver necrosis and hepatomas were noted after NCI strain A mice were treated with carbon tetrachloride for 4 months (Eschenbrenner and Miller, 1946). Treatment with CCl_4 was started when the mice were 3 months of age and was terminated when they were 7 months old. Groups of five male or female mice were administered carbon tetrachloride by gavage at 0, 1200, 2400, 4800 or 9600 mg/kg bw. Dose schedules were either 120 doses in 119 days (120/119) i.e., daily, or 30 doses in 116 days (30/116) i.e., 4-day inter- vals. Animals were necropsied at 8 months (30 days after

TABLE 11-7

Hepatomas in Male and Female Strain A Mice Given CCl_4 via Stomach Tube[a]

mℓ of CCl_4/g bw 30 doses	Interval Between Doses (days)					Total Excluding Interval 1
	1	2	3	4	5	
16×10^{-4}						
Male	0/6[b]	5/7	6/6	5/8	2/4	
Female	0/6	3/5	2/6	6/7	4/5	33/48
8×10^{-4}						
Male	0/6	3/6	5/6	5/8	3/4	
Female	0/6	2/6	3/6	6/7	5/5	32/48
4×10^{-4}						
Male	0/6	0/6	4/6	6/8	2/4	
Female	0/6	3/6	4/6	3/7	3/4	25/47
2×10^{-4}						
Male	1/6	0/6	4/6	5/5	2/4	
Female	0/6	1/6	2/6	7/10	1/5	22/48
1×10^{-4}						
Male	0/6	1/6	4/6	7/8	1/4	
Female	0/6	0/6	3/6	5/7	2/5	23/48
0						
Male	0/3	0/3	0/3	0/2	0/2	
Female	0/3	0/3	0/3	0/3	0/3	0/22

[a]Source: Adapted from Eschenbrenner and Miller, 1943

[b]No. hepatomas/no. mice

cessation of administration). All mice were given one additional dose of the solution 24 hours prior to necropsy. Note that mice in the two grouos at each dose level were administered the same total amount of CCl_4 over the same period of time, but with a variation in the number of doses into which the total amount was divided, and therefore in the size of each dose. The doses for mice in group one (120/119) were previously determined as being "necrotizing" and "non-necrotizing" and in group two (30/116) as "only necrotizing". Two control groups of mice received 0.02 or 0.005 mℓ olive oil/g bw/dose. The incidence of hepatomas and necrotic lesions is noted in Table 11-8.

At the three highest doses on the 120/119 dose schedule, a 100% incidence of hepatomas was observed. At the highest dose, the mice had numerous hepatomas of up to 1 cm in diameter. At the two intermediate doses the neoplasms were less frequent and smaller in size. No hepatomas were observed in the 1200 mg/kg group. The incidence of hepatomas was decreased in the animals on the 30/116 schedule as compared to those on the 120/119 schedule. At the 1200 mg/kg dose, however, very small hepatomas were detected by microscopic examination in two males. Mice given olive oil did not have tumors.

The presence or absence of hepatomas and of hepatic necrosis was determined. When necrosis of the liver was found in mice with tumors, necrosis was not observed in the hepatomas. The localization of necrosis after chronic administration of CCl_4 did not appear to follow a definite pattern, in contrast to the regular pattern of centrilobular necrosis seen after a single dose was administered to strain A mice.

In summary, mice receiving "non-necrotizing" doses of CCl_4 developed as many, if not more, tumors of the liver than mice given "necrotizing"

TABLE 11-8

Susceptibility of Strain A Mice to Liver Necrosis and the Incidence of Hepatomas 30 Days After 120 or 30 Doses of Carbon Tetrachloride[a,b]

		Carbon Tetrachloride Dose														
		9600 mg/kg			4800 mg/kg			2400 mg/kg			1200 mg/kg			0 (olive oil)		
Sex	Dose Conditions (doses/days)	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
F	120/119	+	0/5	5/5	+	0/5	5/5	±	0/5	5/5	-	0/5	0/5	-	0/5	0/5
M	120/119	+	0/4	4/4	+	0/5	5/5	±	0/5	5/5	-	0/5	0/5	-	0/5	0/5
F	30/116				+	2/4	2/4	+	2/5	3/5	+	0/5	0/5	-	0/5	0/5
M	30/116				+	4/4	3/4	+	3/5	4/5	+	0/5	0/5[c]	-	0/5	0/5

[a]Source: Adapted from Eschenbrenner and Miller, 1946

[b]Based on gross and microscopic examination

[c]Two animals showing no tumor on gross examination were found each to have a very small hepatoma on microscopic examination of a random section of the livers.

A = Probable initial liver necrosis (+ = presence, - = absence)
B = Observed final liver necrosis
C = Hepatoma incidence

doses despite the fact that equal amounts of CCl_4 were administered. Mice given 1200 mg/kg did not have gross tumors; most mice receiving either 2400, 4800 or 9600 mg/kg bw did have tumors.

NCI (1976) performed a bioassay on trichloroethylene in which CCl_4 was used as the positive control. Tests were done using $B6C3F_1$ male and female mice (35 days of age, 50 per group). Treatment by oral gavage 5 times per week occurred for 78 weeks. Surviving mice were sacrificed at 92 weeks from the start of the study. The doses of CCl_4 were 1250 or 2500 mg/kg bw for mice of both sexes. There were 20 control mice of each sex that were given corn oil only. A necropsy was performed on all mice along with complete histological examinations.

Most male and female mice treated with CCl_4 were dead by 78 weeks (Table 11-9). Median survival times were 63 and 72 weeks for the males given the low and high doses, respectively and 58.5 and 68.5 weeks for the females given the low and high doses, respectively. Hepatocellular carcinomas were found in practically all mice receiving CCl_4, including those dying before termination of the test (Table 11-10). The first carcinomas were observed in low dose female mice at 16 weeks, in high dose female mice at 19 weeks, in high dose males at 26 weeks, and in low dose males at 48 weeks, compared to 72 weeks for pooled control males and 90 weeks for pooled control females. Cystic endometrial hyperplasia occurred in both control and treated female mice. Thrombosis of the atrium of the heart was seen in 9 of 41 high dose female mice (22%), all of which died with carcinomas of the liver. Some liver toxicity occurred, identified as cirrhosis, bile duct proliferation, toxic hepatitis and fatty liver; however, these cases were few in number. In summary, this study found carbon tetrachloride to be highly carcinogenic for liver in mice. It is used by the Carcinogen Assessment Group (CAG) of the U.S. EPA in determining the carcinogenic risk esti-

TABLE 11-9

Survival of B6C3F$_1$ Mice Treated with Carbon Tetrachloride[a]
(CCl_4 administered by oral gavage)

Dose	Initial	78 Weeks	91-92 Weeks
MALE			
Control			
Matched	20	13 (65%)	7 (35%)
Pooled	77	53 (69%)	38 (49%)
Low Dose[b]	50	11 (22%)	0 (0%)
High Dose[c]	50	2 (4%)	0 (0%)
FEMALE			
Control			
Matched	20	18 (90%)	17 (85%)
Pooled	80	71 (89%)	65 (81%)
Low Dose[b]	50	10 (20%)	0 (0%)
High Dose[c]	50	4 (8%)	1 (2%)

[a]Source: NCI, 1976

[b]1250 mg/kg bw

[c]2500 mg/kg bw

TABLE 11-10

Incidence of Hepatocellular Carcinomas in Mice Treated with Carbon Tetrachloride[a] (CCl_4 administered by oral gavage)

Dose	Hepatocellular Carcinomas
MALE	
Pooled Controls	2/19 (11%)
Low Dose[b]	49/49 (100%)
High Dose[c]	47/48 (98%)
FEMALE	
Pooled Controls	1/20 (5%)
Low Dose[b]	40/40 (100%)
High Dose[c]	43/45 (96%)

[a]Source: NCI, 1976

[b]1250 mg/kg bw

[c]2500 mg/kg bw

mate for CCl_4, in spite of the high doses but justified by the marked effects.

Confer and Stenger (1966) studied nodules in the livers of C3H mice after long-term CCl_4 administration. Twenty-five male mice, 5 weeks of age, received rectal instillation of 0.1 mℓ of a 40% solution of carbon tetrachloride dissolved in olive oil two times a week for 20 to 26 weeks. Ten control mice were given only olive oil. Fourteen mice were killed 9 days after the last treatment, and the remaining mice were killed at periods of 3 to 37 weeks. The livers were examined by light and electron microscopy. Five of the 14 mice (36%) killed after 9 days, and 8 of 11 mice (73%) killed later, developed hyperplastic hepatic nodules. Cirrhosis was not observed in the liver.

In summary, mice given carbon tetrachloride by rectal instillation had hyperplastic nodules that persisted after the discontinuation of the chemical, but did not develop cirrhosis of the liver. Confer and Stenger (1966) proposed hyperplastic nodules, as observed in their study, as precursors of liver carcinomas.

In 1942, using CCl_4, Edwards and Dalton studied the induction of cirrhosis of the liver and hepatomas in mice. They investigated the outcome of high dose, low dose and limited treatment. For high dose administration, strain C3H male mice, male and female strain A mice, male and female strain Y mice and strain C female mice were used. They were started on the study at 1 to 5 months of age.

In one experiment, a dose of 0.1 mℓ of a 40% solution of CCl_4 (with no impurities) in olive oil was administered by stomach tube two or three times per week. The total number of treatments varied from 23 to 58. In order to study any early pathologic changes, a number of mice were killed

after receiving 1 to 23 doses. In another experiment, male mice were given 0.1 mℓ of olive oil 2 or 3 times a week for 39 to 62 doses.

Animals were killed at 1 year of age or younger by cervical dislocation. Subcutaneous transplants of tumor tissue were made by the trocar technique into mice of homologous strains. Special histological techniques were used to examine a number of primary and transplanted tumors. These include techniques for the presence of fat, glycogen or alkaline phosphatase and those for studying the mitochondria and Golgi apparatus.

Hepatomas were observed in 88% of C3H male mice treated with CCl_4, whereas they occurred in 4% of untreated mice of the same age and strain. Tumors of the liver developed in 60% of male and female Y strain mice, whereas only 2% were seen in untreated mice of that strain. Liver tumors were seen in 98% of strain A mice of both sexes, whereas only 2% of these mice developed the tumor spontaneously. Hepatic tumors were found in 83% of C strain females, compared with 0% of untreated mice of the same age and strain. The hepatic tumors observed in this study were usually multiple -- as many as 10 occurring in one liver. Results of both the treated and control groups are given in Tables 11-11 and 11-12. Tumors did not appear to have been induced in any of the other organs.

Low dose administration (0.1 mℓ of 5% CCl_4 in olive oil - 0.005 mℓ) occurred three times weekly by stomach tube to 58 strain A female mice, 2.5 months of age, for 2 months. Mice were necropsied 2 days to 4.5 months after the last treatment. Hepatomas were present in 41 mice (71%), and some mice had cirrhosis of the liver.

The total dose (0.125 to 0.145 mℓ CCl_4) is comparable to the total dose of 0.120 mℓ CCl_4 in the experiment in which mice were given treatments of 0.04 mℓ each. The tumors of the liver were morphologically similar in both studies.

TABLE 11-11

Hepatomas in Male and Female Mice Given Carbon Tetrachloride (0.04 mℓ 2-3x weekly) by Stomach Tube*

Strain	Age (months)	Males	Females	Both
C3H	6-10	126/143 (88%)	-	-
Y	4-12	-	-	9/15 (60%)
C	6-7	-	34/41 (83%)	-
A	4-12	-	-	161/164 (98%)

Hepatomas in Untreated Male and Female Mice

Strain	Age (months)	Males	Females	Both
C3H	8-11	2/50 (4%)	-	-
C3H	12-19	86/320 (27%)	-	-
Y	10-16	-	-	3/129 (2%)
C	13-24	-	0/150 (0%)	-
A	4-8	-	-	0/400 (0%)
A	12-16	-	-	8/400 (2%)

*Source: Edwards and Dalton, 1942

TABLE 11-12

Hepatomas in Male Mice Given Olive Oil by Stomach Tube*

Strain	Age (months)	Incidence
C3H	10-11	4%
C	12	0%
A	5-12	0%

*Source: Edwards and Dalton, 1942

Limited treatment involved strain A female mice, 2 months of age. There were 21 to 62 mice in three treatment groups. The CCl_4 used was dissolved in olive oil, the volume of the mixture administered amounting to 0.1 mℓ. The mice were given 1 to 3 treatments. The doses, which were hepatotoxic, were 0.04, 0.01 or 0.005 mℓ CCl_4. Eleven mice received olive oil only. The mice were necropsied 2 to 12 months after the start of the study.

Tumors of the liver were not found in these mice. There was pigment in Kupffer cells, occasional foci of basophilic debris, and an increase in connective tissue and reticulum.

In summary, carbon tetrachloride induced significant numbers of tumors of the liver, as well as cirrhosis, in three strains of mice (Edwards and Dalton, 1942).

Since successful transplantation is frequently considered to be a criterion of neoplasia, Leduc and Wilson (1959) attempted to transplant CCl_4-induced tumors of the liver in mice. At first

> "numerous failures to establish a transplantable CCl_4-induced hepatoma supported the idea that, if transplantability is a criterion, the nodules might be hyperplastic but not neoplastic. Subsequently, however, several such nodules were successfully transplanted from a host that was allowed to live for a long period after the CCl_4 administration ceased" (Leduc and Wilson, 1959).

Male mice of the BUB strain were used. Spontaneous hepatomas have not been found in this strain, up to its 40th generation. Carbon tetrachloride was administered by stomach tube in doses of 0.1 mℓ of a 40% solution in olive oil (0.04 mℓ CCl_4) per treatment. Carbon tetrachloride was given 3 times a week for a total of 45 to 66 doses. About one-third of the mice were given three daily intravenous i.v. injections of 0.2 mℓ of thorotrast before CCl_4 administration was started. As discussed by Leduc and Wilson

(1959), thorotrast is useful in the detection of hepatomas but has been involved (as indicated by other investigators), in tumor production.

The first-generation tumor transplants were made subcutaneously. Subsequently, both subcutaneous and intrasplenic transplants were made. Under light ether anesthesia, implants of tumor into the spleen were made by an incision through the dorsal body wall. The spleens were examined periodically by laparotomy.

Hepatomas did not develop in 20 control mice given thorotrast only. Hepatomas did occur in CCl_4-treated mice that were free of thoratrast.

The CCl_4 hepatomas (5 of 7) that were successfully transplanted differed from those that did not grow in new hosts because a longer time period elapsed between CCl_4 administration and tumor transplantation. The five successful transplants were obtained from a single host killed 8 months after the last treatment, whereas those that did not grow were transplanted 11 weeks or so after the last treatment. The authors concluded that chronic CCl_4 injury to the liver induces the development of both hyperplastic nodules and hepatomas based on their results. They found the livers of CCl_4-treated mice to be cirrhotic with numerous hyperplastic nodules.

Weisburger (1977) did a series of carcinogenicity studies on halogenated hydrocarbons using both mice and rats. Carbon tetrachloride was used as the positive control. The compounds were tested by oral intubation in 200 Osborne-Mendel rats and 200 $B6C3F_1$ mice of both sexes. Maximum dose levels were 2500 mg/kg for both male and female mice and 100 mg/kg and 150 mg/kg for male and female rats, respectively. A high yield of both hepatocellular carcinomas and adrenal tumors was seen in male and female mice.

Neoplastic nodules and a few carcinomas of the liver were observed in the rats, which, as the author points out, was lower than anticipated (presumably based upon earlier findings). Some of the results are provided in Table 11-13.

This study can be criticized in that the report is poorly written by omitting some important information. Only the maximum tolerated dose is reported for each species and each sex. The low dose is not given. The percent survival is also not reported, nor are final numbers in each group. Finally, tumor type is not specific. The study, however, is included here since it is a part of the literature dealing with the carcinogenicity of CCl_4. However, it is recommended that the data on CCl_4 given in the NCI (1976) bioassay for trichloroethylene be used instead.

11.3. HAMSTERS

Hamsters have not been studied as extensively as have rats and mice. There has been only one report of the induction of tumors in hamsters by CCl_4.

Della Porta et al. (1961) orally administered carbon tetrachloride to Syrian golden hamsters as a part of a larger investigation of the response of this species to carcinogens that induced neoplasms of the liver in other species. Ten female and 10 male Syrian golden hamsters, 12 weeks old, were used. Males weighed an average of 109 g and females 99 g. The treatment consisted of weekly administration by stomach tube of a 5% solution of CCl_4 in corn oil for 30 weeks. No information was given on control animals. During the first 7 weeks, 0.25 mℓ of the solution containing 12.5 μℓ CCl_4 was given each week. This dose was then reduced to 0.125 mℓ and contained 6.25 μℓ of CCl_4. After this treatment, the survivors were kept under observation for 25 additional weeks and then killed.

TABLE 11-13

Incidence of Tumors in Rats and Mice Ingesting Carbon Tetrachloride*

Species	Tumor Type	Male			Female		
		Control	Low	High	Control	Low	High
Rat	Adrenal tumors	1	1	3	0	1	0
	Thyroid-adenoma and carcinoma	1	1	1	2	2	4
	Liver-neoplastic nodule	0	9	3	1	11	9
	Liver hepatocellular carcinoma	0	2	2	1	4	2
	Total tumors	12	29	26	17	46	24
	# animals examined	20	49	50	20	50	49
	# animals with tumors	7	21	24	10	34	20
Mouse	Liver hepatocellular carcinoma	3	49	47	1	40	43
	Adrenal adenoma and pheochromocytoma	0	28	28	0	15	10
	Total tumors	4	81	75	3	56	54
	# animals examined	18	49	48	18	42	45
	# animals with tumors	4	49	47	3	40	43

*Source: Weisburger, 1977

Detailed histopathological examinations of all hamsters were conducted, except for one female lost through cannabalism at the 28th week.

Weights of the hamsters varied irregularly during the period following treatment. In general, the weights increased. Females weighed an average of 114 g and males 118 g. One female died at the 10th week of treatment; three females and five males died or were killed between the 17th and the 28th week. Three females died at weeks 41, 43 and 54. The surviving three females and five males were killed at the end of the 55th week.

Hamsters dying during the treatment and at the 41st week had cirrhosis, as well as hyperplastic nodules that were two to several layers thick. The cells showed irregularities in the shape, size and staining qualities of their cytoplasm and nucleus, with an uneven distribution of glycogen.

All of the animals, five males and five females, dying or killed 13 to 25 weeks after the end of the treatment, had one or more liver-cell carcinomas (a total of 22 tumors: 12 in the 5 females and 10 in the 5 males). No mention was made of toxicity in these animals. Liver cell carcinomas were not found in the other animals which died before week 43.

Transplantation efforts were not successful. The authors note in their discussion that this negative result deserves further investigation since "many other tumors of hamsters have been successfully transplanted to non-inbred hamsters in this and other laboratories" (Della Porta et al., 1961).

In summary, Syrian golden hamsters appear sensitive to the carcinogenic effects of carbon tetrachloride. Although the number of animals in this study was small, the authors considered the results to be significant because the reported historical control incidence of hepatic tumors in hamsters was 0/254. Hyperplastic nodules appeared during treatment, and carcinomas appeared after CCl_4 administration had been discontinued, which

suggests that the nodules or benign tumors were precursor lesions for carcinomas. It should be noted that this study is the only report found in the available literature, of the induction of tumors in hamsters by CCl_4.

In concluding this section, it should be noted that some of the research reported suggests that hepatomas occur only after liver necrosis and fibrosis have occurred (Edwards, 1941; Edwards and Dalton, 1942; Della Porta et al., 1961; Reuber and Glover, 1967, 1970). The results have been interpreted to mean that "as far as the liver is concerned, hepatoma is an occasional consequence of the induction of postnecrotic cirrhosis and that CCl_4 is not a direct liver carcinogen" (Louria, 1977). The results reported by Eschenbrenner and Miller (1946), however, refute Louria's statement. These authors concluded that if carbon tetrachloride is, in fact, a carcinogenic agent, tumors should be obtained with non-necrotizing doses. As discussed earlier in this chapter, their series of experiments examining the issue, revealed:

> While it was found that a correlation exists between the degree of liver necrosis and the incidence of hepatomas in relation to dose, the use of a graded series of necrotizing and non-necrotizing doses indicated that repeated liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of tumors with carbon tetrachloride (Eschenbrenner and Miller, 1946).

The small number of animals used in this study must be noted.

A list of authors addressing the issue of liver necrosis induced by carbon tetrachloride is provided in Table 11-14.

11.4. HUMANS

11.4.1. Case Reports. As mentioned in the section dealing with human toxicity, in many of the case reports the investigators present the data in narrative form. Although interesting, these type of data are not suitable to quantitative analysis since numbers are not adequately presented.

TABLE 11-14

Studies in Which Liver Necrosis was Induced
Using Carbon Tetrachloride

Species--Route	Reference
Mice -- gavage	Edwards, 1941
Mice -- ingestion	Edwards and Dalton, 1942
Mice -- ingestion	Eschenbrenner and Miller, 1946
Hamsters -- ingestion	Della Porta et al., 1961
Rats -- subcutaneous injection	Reuber and Glover, 1967
Human -- ingestion	Hashimoto et al., 1968
Rats -- subcutaneous injection	Reuber and Glover, 1970

Furthermore, there usually are a number of uncontrolled variables (alcohol intake, age, simultaneous exposures) or unknown variables (exposure amount) making it difficult to attribute the outcome solely to the CCl_4 exposure. Despite these limitations, they are important since they can either support or challenge the experimental animal studies and can aid qualitatively in the extrapolations from animals to humans.

The carcinogenic effect of exposure to carbon tetrachloride in humans has been suggested in a number of case reports by physicians. One of these was reported by Tracey and Sherlock (1968). A 59-year-old man with a history of moderate alcohol consumption returned from a cocktail party and noticed the vapor of carbon tetrachloride used to clean a rug in his apartment earlier that evening. Five days later he developed nausea, vomiting and diarrhea and within 10 days of exposure he developed jaundice. The patient recovered following a long and complex hospitalization and was discharged after 9 weeks. Four years after hospitalization for jaundice, he was found to have a smooth, enlarged, nontender liver. He denied alcohol consumption within the intervening period. Three years after this checkup, the patient was readmitted with a history of nausea, vomiting and diarrhea. He again denied ingestion of alcohol. A liver biopsy was diagnosed as hepatocellular carcinoma. Postmortem examination revealed the liver to be extensively involved with tumor. Little normal liver tissue remained.

Aside from the acute exposure to carbon tetrachloride 7 years before diagnosis of cancer, the patient's possible additional exposure to this and other toxic chemicals was not reported. No medical history was given for the 3 years before final diagnosis.

Other case reports of human neoplasms developing after exposure to carbon tetrachloride have appeared. In one, a woman developed modular cirrhosis of the liver followed by cancer of the liver after exposure to carbon tetrachloride, and died 3 years after the first exposure (Johnstone, 1948). However, she had suffered from periodic jaundice for 5 years prior to the CCl_4 exposure. In a second, a fireman developed cirrhosis and an "epithelioma" of the liver 4 years after acute carbon tetrachloride intoxication (Similar et al., 1964). In none of the cases could a causal link between carbon tetrachloride exposure and development of neoplasms be established.

11.4.2. Studies. A study by Capurro (1979) reports a series of cancer cases in a rural valley polluted by vapors from a solvent recovery plant. Due to its lack of specificity and questionable statistical methods, the study is of limited value. However, in an effort to be complete, it is described and critiqued. Odor and pollution problems existed in this valley from 1961 to 1971. A variety of solvents including carbon tetrachloride were identified in the air. Blood tests were done on 24 residents; solvents were detected in the blood of all those tested. A list of solvents detected in the blood is not given but those "most easily detected" (as cited by the author) were: benzene, chloroform, methyl isobutyl ketone and trichloroethylene. Levels were not reported.

A study population was defined consisting of 117 Caucasians who lived within 1.5 km^2 of the plant during the appropriate time for greatest exposure. These persons were followed for a 6-year period.

Ten cancer cases occurred during this time period including two cases that were not residents. The author's analysis focuses on four cases of lymphoma (2 lymphosarcoma, ICD 200.0 and 2 reticulum cell sarcoma ICD 200.1). All four of these cases had a history of work in the paper mill

which preceded the solvent recovery plant at the same location. The author reports that a study of paper mill workers (Milham, 1976) showed a proportionate mortality ratio [PMR = (observed deaths/expected deaths) x 100] of 1.8 for lymphosarcoma, but no significant increase of reticulum cell sarcoma. However, as Capurro (1979) reports, "the excess lymphomas observed by us is far above the one described by Milham". A mortality ratio indicating a 160-fold increase for these two cancers (ICD 200.0 and 200.1) was calculated. However, it should not be reported due to the errors in its calculation. Three observed deaths are used, rather than four, with no explanation and the study population is rounded off to 120 instead of using the defined 117. The ratio is not standardized by age or sex, nor is it described as race-specific (all Caucasians). The assumption is made that since the age distribution of the study population is comparable to that of the nation, age standardization is not necessary. However, the study population is then compared to the population of the State of Maryland rather than the nation. The discussion of lymphomas diagnosed in the county and in the area surrounding the plant lacks important information on population distribution, time period and comparison group as well as methods used. Thus, in lieu of these deficiencies, the conclusion that the incidence of malignant lymphoma is abnormal in the area exposed to solvent vapors is not supported.

In a preliminary study designed to determine if occupational exposure to carbon tetrachloride, trichloroethylene and tetrachloroethylene resulted in increased mortality, Blair et al. (1979) studied causes of death in 330 laundry and dry cleaning workers. Sex, race and age along with underlying and contributing causes of death were abstracted from death certificates.

The underlying cause was classified according to the International Classification of Diseases, Seventh Revision, by a trained nosologist. The control standard was the age, race, sex and cause specific distribution of U.S. deaths from the same time period. The PMR for all malignant neoplasms was 128 (100 would signify no difference from the control) and statistically significant (χ^2 test, $p<0.05$). The excess deaths consisted of lung, cervical and liver cancers, and leukemia. The authors note that the lung and cervical cancer excess may reflect the lower socioeconomic status of these workers. The slight excess of liver cancer is consistent with bioassay studies showing liver abnormalities. The authors conclude that epidemiologic studies of this occupational group are warranted due to the increased risk observed.

Finally, increases in certain types of skin cancers can be attributed to increased atmospheric CCl_4. As explained in Chapters 5 and 6, CCl_4 may result in an overall reduction of stratospheric ozone, eventually leading to increased UV-B radiation. It is estimated that a 2 to 5% increase in basal cell skin cancer will occur per 1% decrease in ozone and that a 4 to 10% increase in squamous cell skin cancer will occur per 1% decrease in ozone (NAS, 1982). The relationship between sunlight and malignant melanoma is not clear at present.

11.5. SUMMARY

11.5.1. Experimental Animals. Carbon tetrachloride has been reported to be carcinogenic in numerous animal studies. Hepatocellular carcinomas have been the neoplasm induced in all species evaluated (rats, mice and hamsters). An increase in adrenal tumors in male and female mice was seen in one study. Hamsters, although only used in one study, have been the most sensitive species studied, followed by mice and then rats. A significant

strain difference has been observed in rats. Females have appeared less sensitive to the chronic toxic effects but more sensitive to the carcinogenic effects of carbon tetrachloride in rats.

11.5.2. Humans. A number of cases of hepatomas appearing in humans years following exposure to CCl_4 have been reported. A study examining the effect of solvent vapors (one of which was CCl_4) on a group of environmentally exposed people concluded the existence of an abnormal incidence of malignant lymphoma. However, it should not be used as evidence of the carcinogenicity of CCl_4 due to the concomitant exposures and poor study techniques. A preliminary epidemiological study of a group occupationally exposed to CCl_4 revealed a slight excess of liver cancer in this group which offers better evidence of human carcinogenesis associated with CCl_4 exposure, and points the way to further research needs.

11.5.3. Conclusion. In conclusion, there is evidence that CCl_4 is a potential human carcinogen based upon the positive findings on mice in the NCI bioassay for trichloroethylene (1976) in which CCl_4 was used as the positive control. Other studies on animals support this conclusion such as the hamster study by Della Porta et al., (1961), the rat studies by Reuber and Glover (1967, 1970) and NCI (1976), and the mice studies by Edwards (1941), Edwards et al. (1942), Edwards and Dalton (1942) and Weisburger (1977). Human data as reported by Blair et al. (1979) also are consistent with this conclusion.

12. SYNERGISM AND ANTAGONISM

12.1. SYNERGISM

A description of the entire clinical picture of the toxicity of CCl_4 should consider the role played by alcohol in the genesis of severe CCl_4 poisoning (von Oettingen, 1964). A number of researchers have reported on this phenomenon (Stevens and Forster, 1953; Kirkpatrick and Sutherland, 1956; Joron et al., 1957; New et al., 1962; Markham, 1967). It has been established that habitual ingestion of alcoholic beverages and also their occasional use may increase the dangers from comparatively moderate exposure (Markham, 1967; Tracey and Sherlock, 1968). This fact is illustrated by reports on simultaneous exposure of abstinent persons and consumers of alcohol to the same concentration with only the latter becoming seriously ill (von Oettingen, 1964).

An example of this was given by Smetana (1939) who reported on three cases of CCl_4 poisoning. In one case, a 35-year-old male dry cleaner/interior decorator (a "steady and heavy drinker") had been cleaning furniture and draperies with CCl_4. Several hours later, he developed the typical acute symptoms of dyspnea, cough and bloody sputum associated with exposure to CCl_4. His condition worsened and he subsequently died. A coworker of his, a teetotaler, had been working in the same room for the same amount of time receiving the same exposure to the CCl_4. The author stated that "although he felt the effect of the exposure and suffered from headache and gastrointestinal distress, he recovered quickly after breathing fresh air" (Smetana, 1939).

Hypotheses have been advanced to rationalize this apparent synergistic reaction between alcohol and CCl_4. Lamson et al. (1928) postulated that the alcohol either caused a greater absorption from the gastrointestinal

tract or a greater penetration into the liver. Smetana (1939) theorized that the reduction of the glycogen store in the hepatic cells of alcoholics may have some role in the greater susceptibility to CCl_4 poisoning. [This latter tneory is supported by the findings that neonates are protected against CCl_4 poisoning and that neonates have an increased concentration of liver glycogen (Bhattacharyya, 1965)].

Traiger and Plaa (1971) investigated the potentiating capacity of aliphatic alcohols on CCl_4 toxicity. Male Swiss-Webster mice were given a single dose by gavage of methanol, ethanol or isopropanol equivalent to 50% of the LD_{50}. After 20 hours, an i.p. injection of 0.0075 mℓ/kg CCl_4 in corn oil was administered. Controls received only the corn oil. Blood samples were analyzed for SGPT activity. All alconols produced increased activity but the effect was most marked among the mice pretreated with isopropanol. The administration of the alcohols or CCl_4 alone did not change the SGPT levels.

In an effort to ascertain the differences of the alcohols in the potentiation of CCl_4, the authors performed more detailed biocnemical studies on male Sprague-Dawley rats. In these experiments, a single dose of ethanol or isopropanol was administered by gavage 3 to 48 hours prior to the i.p. injection of 0.1 mℓ/kg CCl_4 in corn oil. Controls received only the corn oil. After 24 hours, the animals were sacrificed and blood and liver aliquots were taken for SGPT, glucose-6-phosphatase (G6PD), bilirubin and hepatic triglyceride analysis. Isopropanol produced a more marked change in the activities of SGPT, G6PD and triglycerides. Isopropanol pretreatment produced hyperbilirubinemia whereas ethanol pretreatment or CCl_4 alone at a 10-fold increase in dosage did not. Also, a 10-fold dosage increase in

CCl_4 alone produced an increased level of SGPT activity; however, it was only approximately 25% of the SGPT activity found among rats pretreated with isopropanol.

Wei et al. (1971) investigated the potentiation of CCl_4 hepatotoxicity by ethanol and cold. This was accomplished by pretreating rats with ethanol and exposing rats to a cold temperature (18 hours at 4°C). Indices of hepatotoxicity were SGPT levels and liver triglyceride levels. In both male and female rats, the SGPT levels increased after both ethanol and cold exposures in response to the CCl_4. The authors postulate that the ethanol releases norepinephrine, which increases the susceptibility of the liver to CCl_4. According to Davis (1934), very obese or undernourished persons suffering from pulmonary diseases or gastric ulcers or having a tendency to vomiting, liver or kidney diseases, diabetes or glandular disturbances are especially sensitive to the toxic effect of CCl_4 (von Oettingen, 1964).

Strubelt et al. (1978) found that carbon tetrachloride-induced liver damage was significantly greater in rats concomitantly exposed to ethanol than in control rats not exposed to ethanol. Male Wistar rats were given either a 5% or 15% ethanol solution as their sole source of fluid (11.4 or 24.9% of total colonies, respectively). Controls were provided with tap water. Following 1, 2 and 3 weeks of exposure, CCl_4 was administered intraperitoneally at a dose of 0.1 mg/kg. Hepatotoxic effects were evaluated by measuring the serum activities of glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT) and sorbital dehydrogenase (SDH) as well as histological investigations.

The potentiation noted was fully developed following 1 week of exposure and was greater in those rats provided a 15% ethanol solution than in the rats provided a 5% ethanol solution, thus appearing to be dose-dependent.

Hasumura et al. (1978) also examined the potentiation of carbon tetrachloride hepatotoxicity by ethanol. They designed their study to determine if hepatic microsomal changes which are secondary to chronic ethanol consumption, play a role in the mechanism of CCl_4 hepatotoxicity. Rats were pair-fed a liquid diet containing ethanol (36% of calories) or isocaloric carbonydrate for a period of 4 to 5 weeks. Carbon tetrachloride, at a dose of 0.5 mℓ/kg, was administered intragastrically 15 hours after ethanol withdrawal. Within 24 hours there was an increase in liver lipids and serum ornithine carbamyl transferase activity and a decrease in the activities of hepatic aminopyrene N-demethylase and glucose-6-phosphatase. These changes were determined to be significantly greater in the ethanol-fed rats versus the control rats indicating *in vivo* potentiation of carbon tetrachloride hepatotoxicity by chronic ethanol consumption, even in the absence of ethanol at the time of exposure (Hasumura et al., 1978).

In an attempt to determine the mechanism of this effect, liver microsomes were incubated with $^{14}CCl_4$ and a NADPH-generating system. The authors found an enhancement of: (1) the covalent binding of ^{14}C to microsomal protein *in vitro* and (2) the biotransformation of $^{14}CCl_4$ to $^{14}CO_2$ *in vitro*. This suggests that ethanol pretreatment stimulates the microsomal formation of an active metabolite of CCl_4 thus, microsomal changes are responsible, at least partially for the increased CCl_4 hepatotoxicity (Hasumura et al., 1978).

In addition to ethanol other compounds have been investigated as to their effect on carbon tetrachloride hepatotoxicity. Curtis et al. (1979a) and Davis and Mehendale (1980) have studied the potentiation of carbon tetrachloride hepatotoxicity by exposure to chlordecone (Kepone).

Curtis et al. (1979a) found greatly potentiated hepatotoxicity reflected in the form of elevated SPGT and SGOT activities between groups of rats which had undergone a 15-day feeding of 0 or 10 mg/kg chlordecone, and a single intraperitoneal challenge of CCl_4. The CCl_4 challenge was either 0, .025, .05, .1 or .2 mℓ/kg. The SPGT and SGOT activities increased in excess of 30-fold and 10-fold respectively in chlordecone-fed rats challenged with CCl_4 of .1 and .2 mℓ/kg. The authors concluded that the data indicate a great potential for the production of severe liver damage resulting from interactions of carbon tetrachloride and chlordecone exposure at levels which may be independently non-toxic.

Davis and Mehendale (1980) conducted a similar study using a single per os administration of chlordecone (5 mg/kg) followed 48 hours later by intraperitoneal administration of CCl_4 (.2 mℓ/kg). Twenty-four hours later the hepatic excretory function of the animals treated with chlordecone and CCl_4 had decreased (20% of controls), while plasma transaminase activities and bilirubin were elevated. Parameters measured or assessed which were not affected by chlordecone pretreatment were: hepatic mixed function oxidase activity, irreversible binding of label from $^{14}CCl_4$ to hepatic protein or lipid, hepatic and renal glutathione concentrations and CCl_4-induced lipid peroxidation of liver tissue measured *in vitro* and *in vivo*. The authors conclude that the mechanism for the enhanced toxicity is still unknown; however, the results suggest the interaction between chlordecone and CCl_4 is a subtle one, not causally involving increased covalent binding of the toxin, increased susceptibility of tissue lipids to peroxidative damage or decreased hepatic glutathione.

Curtis et al. (1979b) investigated the effect of pre-exposure to 50 mg/kg photomirex on carbon tetrachloride hepatotoxicity in rats. Photomirex

is a photodegradation product of the insecticide mirex and is also a structural analog of chlordecone. The photomirex/CCl_4 interaction resulted in liver hypertrophy, 7- and 8-fold elevations in SGOT and SGPT over the control rats and rats treated with photomirex alone, and considerable centrilobular necrosis. Liver weight, SGOT and SGPT were unaffected by the CCl_4 challenge alone, therefore pre-exposure to photomirex does potentiate CCl_4 hepatotoxicity as does chlordecone, a structural analog.

In a review article, Falk (1976) reported on the effects of different chemicals on carbon tetrachloride toxicity. A summary of this review is presented here. A low protein diet fed to rats reduced the hepatic microsomal hydroxylase activity and dramatically reduced the toxicity of carbon tetrachloride. The LD_{50} of CCl_4 was 14.7 mℓ/kg on the protein free diet compared to 6.4 mℓ on a regular diet. When the rats were pretreated with phenobarbitaol the LD_{50} was 0.5 mℓ/kg.

Benzo(a)pyrene (BAP) in conjunction with carbon tetrachloride has been shown to enhance tumor production in laboratory animals (Kotin et al., 1962; Proetzel et al., 1964) while CCl_4 alone was found to produce no sarcomas at all. Weisburger et al. (1965) found that treating rats with acetylaminofluorene (AAF) produced an increase in hepatomas in female rats, from 0% without to 81% with CCl_4. In males, the increase was 73% to 100%. A change in metabolism of AAF toward greater N-hydroxylation on CCl_4 treatment was found to be the reason for the enhanced tumor incidence (Weisburger and Weisburger, 1963).

Kluwe et al. (1980) found that CCl_4 produced liver damage, as represented by increased levels of SGOT in male rats fed with 100 mg/kg polybrominated biphenyls (PBB) or 200 mg/kg polychlorinated biphenyls (PCB) 28 days before injection of CCl_4. The liver damage was greater in the rats

fed PBB than in those fed PCB which in turn showed greater damage than the control rats. Functional renal damage was produced by CCl_4 and several other solvents such as TCE and 1,1,2-trichloroethane. CCl_4-induced renal dysfunction was found to be potentiated by PBB but not PCB.

Dietz and Traiger (1979) found that pretreatment of rats with either 2-butanone or 2,3-butanediol markedly enhanced the hepatotoxic response to CCl_4 as measured by SGPT and hepatic triglycerides. The administration of CCl_4 and quinalphos (an insecticide) was found to cause morphological changes in the liver, kidney and testes of male rats (Dikshith et al., 1980). The authors suggest that pretreatment of CCl_4 made the animals susceptible to quinalphos.

12.2. ANTAGONISM

As to the antagonistic compounds associated with CCl_4, Hafeman and Hoekstra (1977) report a protective effect of dietary vitamin E, selenium (Se) and methionine against lipid peroxidation induced by CCl_4. In the first of three experiments, 21-day-old male weanling rats were fed a diet deficient in vitamin E and Se and low in methionine. Dietary supplements of these three variables were administered either alone or in combination. To assess the effect of CCl_4, they monitored lipid peroxidation by the evolution of ethane, an auto-oxidation product of ω-3-unsaturated fatty acids. To study the effect of increasing dietary ω-3-unsaturated fat on CCl_4-induced ethane evolution, cod liver oil (CLO), which is rich in ω-3-unsaturated fat, was substituted for lard. A dose of 2 mℓ CCl_4/kg bw was administered via i.p. injection. The investigators found that in all dietary groups, CCl_4 stimulated ethane evolution. However, among mice given dietary supplements of vitamin E, Se and methionine, ethane evolution was reduced 17, 26 and 39%, respectively. The substitution of CLO for lard

in the diets resulted in a 6-fold increase in ethane evolution. If only CCl_4-induced ethane evolution is considered, only dietary supplements of vitamin E and Se resulted in a statistically significant ($p<0.05$) reduction of ethane evolution. It was also noted that among rats given CCl_4 with no supplements there was pronounced mortality which correlated well with ethane evolution. The authors conclude that the toxicity of CCl_4 was decreased in correlation with ethane evolution. Thus, vitamin E, Se and methionine protected against CCl_4-induced lipid peroxidation, probably by maintaining intracellular glutathione and glutathione peroxidase.

In two other experiments, the authors sought to determine the effectiveness of each protective factor alone or in combination and to estimate peroxidation rates in rats in which CCl_4 caused early mortality. In these experiments, a dose of 1 mℓ CCl_4/kg bw was administered via i.p. injection. Results indicate that vitamin E, Se and methionine supplements in all combinations protect against CCl_4 toxicity and that supplements of either vitamin E or Se, or both with methionine, completely prevented CCl_4-induced mortality.

Reserpine, carbon disulfide and diethyldithiocarbamate reportedly diminish the toxic effects of carbon tetrachloride on liver in experimental animals. The mechanisms are unknown, but some of the investigators have speculated that metabolism via the microsomal enzyme system may be involved (Douglas and Clower, 1968; Seawright et al., 1980; Siegers et al., 1978). Chlorpromazine prevented liver necrosis from carbon tetrachloride in short-term rat experiments without affecting lipid peroxidation or binding of carbon tetrachloride-reactive metabolites (not identified), but it also lowered body temperatures. The investigators were of the opinion that chlorpromazine only delayed the onset of liver necrosis (Marzi et al., 1980). Cagen

and Klaassen (1980) reported that the release of alanine aminotransferase and aspartate aminotransferase into plasma 24 hours following various doses of CCl_4 was markedly lower in rats pretreated for 3 days with zinc chloride (150 µmol/kg/day) than in control rats. Administration of zinc chloride solution was found to increase hepatic concentrations of metallothionein. The authors suggest that metallothionein may protect against CCl_4-induced liver damage by sequestering reactive metabolites of CCl_4.

Miknail et al. (1978) examined the protective effect of adenosine monophosphate (AMP) on CCl_4 toxicity. Three groups of male and female adult rats were studied: a control group, a group treated with 0.5 mℓ of a 1:1 mixture of CCl_4 in mineral oil/100 g bw via intraperitoneal injection and a group treated with 30 mg AMP via intraperitoneal injection 1/2 hour prior to treatment with CCl_4. The CCl_4 resulted in elevated serum iron, copper, zinc, potassium, sodium and calcium 24 hours after administration. However, pretreatment with AMP led to a normalization of the levels of serum iron, copper and zinc while there were no changes in serum calcium, magnesium, potassium and sodium levels as compared to the CCl_4-treated group. The normalization of zinc may be due to the action of AMP on hormone secretion. Thus, the authors conclude that the normalization of iron and copper may be due to some protective effect of AMP on the liver (Miknail et al., 1978).

Additional work is reported in the literature dealing with antagonism but will only briefly be mentioned here. Kieczka et al. (1981) determined that an oxidized catechol metabolite rather than the catechol molecule itself may be responsible for the enzymatic inhibition of the activation step of CCl_4 or may interfere with reactions of the $\cdot CCl_3$ radicals formed. The administration of chloramphenicol early in CCl_4 intoxication

prevents lipid peroxidation of endoplasmic reticulum memoranes in rats (Dolci and Brabec, 1978). Dzhioiev and Balanski (1974) found tnat CCl_4 injected prior to the administration of urethane reduced the incidence of adenomas by 32 and 47%.

12.3. SUMMARY

Numerous substances have been shown to synergistically affect carbon tetracnloride toxicity. Ethanol has been shown to potentiate CCl_4 toxicity even when the ethanol consumption had taken place prior to exposure. This effect has been documented in case studies and more recently quantified in animal studies. Several other environmental pollutants such as Kepone, PBB and PCB have been shown to potentiate CCl_4 toxicity at doses where ooth substances are not considered toxic. CCl_4 toxicity nas been shown to be inhibited by several compounds such as chloramphenicol and catechol.

13. REGULATIONS AND STANDARDS

13.1. WATER

13.1.1. Ambient Water. The U.S. EPA recently announced availability of 64 ambient water quality criteria documents and associated criteria (45 FR 79318). The criteria associated with the carbon tetrachloride document were 4.0, 0.4 or 0.04 µg/ℓ based on estimated human lifetime cancer risks of 10^{-5}, 10^{-6} or 10^{-7}, respectively. These criteria were derived based on the assumption of a daily contaminated water intake of 2 ℓ and contaminated fish intake of 6.5 g per person. Criteria associated with only the consumption of 6.5 g of contaminated fish were 69, 6.9 or 0.69 µg/ℓ, respectively.

13.1.2. Drinking Water. The NAS recommended "Suggested No-Adverse-Response Levels" (SNARLs) for carbon tetrachloride in drinking water (NAS, 1980). The recommended 24-hour value was 14 mg/ℓ based on data which indicated a toxic effect to the liver of rats 5 hours after one exposure to carbon tetrachloride at 400 mg/kg bw, a 1000-fold safety factor, and an assumed daily water consumption of 2 ℓ for a 70 kg adult. NAS recommended a 7-day SNARL of 2 mg/ℓ based on the assumed cummulative effects of carbon tetrachloride after repeated daily exposure (i.e., 14 mg/ℓ ÷ 7 = 2 mg/ℓ). NAS did not recommend a "chronic exposure" SNARL because carbon tetrachloride is a carcinogen in some animal species.

The Office of Drinking Water (ODW) of the U.S. EPA has recommended draft SNARLs for carbon tetrachloride. The 1-day value is 0.2 mg/ℓ based on data which indicate adverse effects in rats after an acute exposure of carbon tetrachloride at 20 mg/kg bw, a 1000-fold safety factor, and an assumed daily water consumption of 1 ℓ for a 10 kg child (Ohanian, 1981). ODW

recommended a draft 10-day SNARL at 0.02 mg/ℓ. A longer-term EPA-SNARL was not developed due to the lack of acceptable data on chronic exposure to this compound.

The differences in the NAS and ODW SNARLs are 3-fold: different data bases were used, the NAS-SNARL was calculated for a 70 kg adult rather than a 10 kg child, and the adult was assumed to ingest 2 ℓ of water/day as compared to 1 ℓ/day for a child. Both methods assume 100% absorption of ingested carbon tetrachloride.

13.2. AIR

The American Conference of Industrial Governmental Hygienists (ACGIH, 1980) has recommended threshold-limit-values (TLVs) for carbon tetrachloride both as a time-weighted average (TWA) and a short-term-exposure limit (STEL) of 30 and 125 mg/m^3, respectively. Previously, ACGIH (1979) recommended values of 65 and 130 mg/m^3, respectively. A concise history of other relevant ACGIH recommendations and accompanying logic can be found in the Ambient Water Quality Criteria Document for Carbon Tetrachloride (U.S. EPA, 1980a).

The Occupational Safety and Health Administration (OSHA), U.S. Department of Labor, adopted the American National Standards Institute (ANSI) standard Z37.17-1967 (ANSI, 1967) as the Federal standard for carbon tetrachloride (29 CFR 1910.1000). This standard is 65 mg/m^3 for an 8-hour TWA exposure, with an acceptable ceiling exposure concentration of 162.5 mg/m^3, and an acceptable maximum peak above the acceptable ceiling concentration for an 8-hour shift of 1300 mg/m^3 for 5 minutes in any 4 hours. This adopted ANSI standard was based on human experience and extensive studies on animals. References cited to support it were Adams et al. (1952), Stewart et al. (1961, 1965), Stewart and Dodd (1964), von Oettingen (1964) and Irish (1963).

The National Institute for Occupational Safety and Health (NIOSH, 1975) recommended a carbon tetrachloride TWA value of 12.6 mg/m^3 for a 10-hour work day, 40-hour week over a working lifetime. This recommendation was based on liver and eye changes found in workers chronically exposed to carbon tetrachloride. In a 1976 revision, NIOSH recommended that the concentration of CCl_4 not be >12.6 mg/m^3 of breathing zone air in a 45 ℓ air sample taken over a period not to exceed 1 hour in duration (NIOSH, 1976)

Inhalation standards for various toxic substances in the working environment of several other countries have been published (International Labor Office, 1970). Carbon tetrachloride inhalation standards are shown in Table 13-1. The USSR values are maximum allowable concentrations (MACs) never to be exceeded. Several countries follow USSR standards; others follow the recommendations of ACGIH.

13.3. FOOD

The National Academy of Sciences (NAS, 1978) reported maximum concentrations of carbon tetrachloride permitted in cooked cereal as 50 µg/kg. This value was derived from a FAO/WHO expert committee in 1972. No other information was found concerning guidelines, criteria or standards for carbon tetrachloride in food.

13.4. SUMMARY

Protective levels for carbon tetrachloride in air in the workplace have been suggested by several countries and by several groups within the United States. The number of suggested protective levels demonstrates the wealth of toxicity data in this area. Protective levels for carbon tetrachloride in water (both drinking and ambient) have recently been suggested by the U.S. EPA and NAS. Only one protective level has been suggested for food: that of cooked cereal. Obviously, more work is needed in this latter area.

TABLE 13-1

Carbon Tetrachloride Inhalation Standards of 11 Countries[a]

Country	Standard mg/m^3	Qualifications
United States		
ACGIH	30[b]	TWA
	125[c]	STEL
OSHA	65	TWA
	162.5	Ceiling exposure concentration
	1300	Maximum peak of ceiling Concentration not to be exceeded for 5 minutes in any 4 hour period.
NIOSH	12.6[b]	TWA
	12.6[c]	45ℓ air/60 minutes
Czechoslovakia	50	MAC
	250	Single short exposure
Finland	160	8 hours continuous exposure
Hungary	20	8-hour average
	100	30 minutes
Japan	10	
Poland	20	
Rumania	50	
UAR and SAR	625	
USSR	20	MAC
Yugoslavia	65	

[a]Source: Adapted from NIOSH, 1975

[b]Recommended standards

14. EFFECTS OF MAJOR CONCERN AND HEALTH HAZARD ASSESSMENT

The assessment of health hazards from carbon tetrachloride requires information which relates specific adverse health effects to dose-exposure conditions. Studies which report the route of exposure, the dose, the duration of exposure, the animal species and the nature of adverse effects are therefore most useful in hazard assessment, which involves the prediction of effects from given environmental or occupational exposure situations. Since the route of intake is often important, experimental studies should use the typical routes of human exposure (i.e., ingestion, inhalation or dermal contact). Therefore, studies based on exposure by intraperitoneal or subcutaneous injection are not included.

The discussion of health effects is organized according to the route of exposure. Within each subsection, the discussion first lists the pertinent acute, subchronic and chronic effects, followed by summaries on reproductive, mutagenic and carcinogenic effects. Specific information on dose, exposure, test animal, effects and references are presented in Table 14-1 for acute, subchronic and chronic studies, in Table 14-2 for reproduction studies, in Table 14-3 for mutagenicity studies, and in Table 14-4 for carcinogenicity studies. Note that these tables summarize the toxicity data and require further analysis before risk estimates can be made for humans.

14.1. PRINCIPAL EFFECTS

Carbon tetrachloride is toxic to humans and animals. Sublethal exposure affects several organs; however, the primary target organs are the liver and the lung. Long-term exposure has resulted in malignant tumors of the liver in three animal species. Several studies have produced satisfactory dose-response information, including estimated "no-effect" levels for humans and four animal species. These studies are classified as satisfactory in that

TABLE 14-1

Dose-Related Toxic Effects of Carbon Tetrachloride
on Humans and Animals

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
Ingestion	Human	0.06 mg	1 day	Creatinine ROR[d] = 0.17	Sonich et al., 1981
	Human	0.11 mg	1 day	Creatinine ROR[d] = 1.25	Sonich et al., 1981
	Human	0.18 mg	1 day	Creatinine ROR[d] = 1.74	Sonich et al., 1981
	Human	0.21 mg	1 day	Creatinine ROR[d] = 3.39	Sonich et al., 1981
	Rat	20 mg/kg	Single dose	Liver cell change	Korsrud et al., 1972
	Rat	40 mg/kg	Single dose	Increased liver fat, increased liver weight, increased liver enzyme levels	Korsrud et al., 1972
	Dog	116 mg/kg	Single dose	NOEL	Gardner et al., 1924
	Rabbit	159 mg/kg	Single dose	Liver necrosis	Gardner et al., 1924
	Dog	797 mg/kg	Single dose	Centrilobular necrosis of liver	Gardner et al., 1924
	Mouse	1600 mg/kg	Single dose	NOEL	Boyd et al., 1980
	Rat	1600 mg/kg	Single dose	Increased liver weight and fatty infiltration, some liver necrosis	Murphy and Malley, 1969
	Rat	3816 mg/kg	Single dose	NOEL	Boyd et al., 1980
	Mouse	4000 mg/kg	Single dose	Reversible widespread lung cell changes	Boyd et al., 1980
	Rat	4000 mg/kg	Single dose	Reversible changes in kidney morphology	Striker et al., 1968

TABLE 14-1 (cont.)

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
	Rat	4000 mg/kg	Single dose	Behavior changes, diverse lung cell changes, altered liver C-450 content	Gould and Smuckler, 1971
	Rat	5088 mg/kg	Single dose	Clara cell lesions in lung	Boyd et al., 1980
	Rat	22 mg/kg	6 wk	NOEL	Alumot et al., 1976
	Rat	40 mg/kg	6 wk	Higher levels of total lipids and triglycerides	Alumot et al., 1976
	Rat	76 mg/kg	6 wk	Depressed weight gain, higher levels of total lipids and triglycerides	Alumot et al., 1976
Inhalation	Human	63 mg/m^3	3 hr	NOEL	Stewart et al., 1961
	Human	309 mg/m^3	1 hr	Decreased serum iron, altered serum transaminase levels	Stewart et al., 1961
	Human	290-650 mg/m^3	2 yr	Reversible nausea, anorexia, vomiting, discomfort	Kazantis and Bomford, 1960
	Rat	300 mg/m^3	24 hr	Reduction in enzyme activity	Merkur'eva et al., 1979
	Rat	65 g/m^3	0.3-4.0 hr	Increased liver and kidney weights	Wong and Di Stefano, 1966
	Mice	71.8 g/m^3	1 hr	Clara cell lesions in lung	Boyd et al., 1980
	Rat	285 g/m^3	0.5 hr	Morphological and cellular changes in lung	Chen et al., 1977
	Rat	6.1 mg/m^3	6 wk[e]	NOEL	Prendergast et al., 1967

TABLE 14-1 (cont.)

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
	Guinea pig	6.1 mg/m^3	90 days[e]	NOEL	Prendergast et al., 1967
	Rat	61 mg/m^3	6 wk[e]	Fatty infiltration and some degeneration of liver	Prendergast et al., 1967
	Guinea pig	61 mg/m^3	90 days[e]	3 deaths (of 15 animals), depressed weight gain, liver damage	Prendergast et al., 1967
	Rat	515 mg/m^3	6 wk[e]	Fatty degeneration of liver, morphological changes in liver and lung	Prendergast et al., 1967
	Guinea pig	515 mg/m^3	6 wk[e]	3 deaths (of 15 animals), weight loss, liver damage, morphological changes in liver and lung	Prendergast et al., 1967
	Rat	14 mg/m^3	5 hr/day, 5 day/wk, 5 mo	Changes in hepatic energy producing processes	Rotenberg, 1978
	Rat	315 mg/m^3	10 mo[e]	Growth stimulation, NOAEL	Smyth et al., 1936
	Guinea pig	315 mg/m^3	10 mo[e]	Mortality (9/24) with median 44 exposures, liver cirrhosis	Smyth et al., 1936
	Monkey	315 mg/m^3	10 mo[e]	Reversible liver degeneration	Smyth et al., 1936
	Rat	630 mg/m^3	10 mo[e]	Liver cirrhosis after 173 exposures	Smyth et al., 1936
	Guinea pig	630 mg/m^3	10 mo[e]	Mortality (16/24) with median 10 exposures	Smyth et al., 1936
	Rat	1260 mg/m^3	10 mo[e]	Liver cirrhosis after 115 exposures	Smyth et al., 1936

TABLE 14-1 (cont.)

Route[a]	Animal	Dose[b]	Exposure	Reported Effects[c]	Reference
	Guinea pig	1260 mg/m^3	10 mo[e]	Mortality (13/24) with median 3 exposures	Smyth et al., 1936
	Monkey	1260 mg/m^3	10 mo[e]	Reversible liver degeneration, depressed weight gain, sciatic nerve damage	Smyth et al., 1936
	Rat	2520 mg/m^3	10 mo[e]	Growth retardation, liver cirrhosis after 54 exposures	Smyth et al., 1936
	Guinea pig	2520 mg/m^3	10 mo[e]	Mortality (19/24) with median 3 exposures	Smyth et al., 1936
Dermal	Guinea pig	1 mL/ 3.1 cm^2	0.3 hr	Epidermal spongiosis and karyopyknosis	Kronevi et al., 1979
	Guinea pig	1 mL/ 3.1 cm^2	16 hr	Pronounced epidermal spongiosis, karyopyknosis and junctional separation, marked hydropic changes in liver, liver necrosis	Kronevi et al., 1979

[a]Oral exposure includes gavage, drinking water and diet.

[b]Oral exposure is in mg CCl_4 or mg of CCl_4/kg body weight. Inhalation exposure is in weight of CCl_4/cubic meter of air. When ppm is supplied by the referenced article, conversion is 6.5 mg/m^3 = 1 ppm. Dermal exposure is volume of CCl_4 per area of skin. These are the reported animal exposure levels and are not human equivalent exposure levels. Therefore, doses are not comparable and may not be directly applicable to the human exposure situation.

[c]The type of effect level (NOEL, etc.) was determined for this document and was not necessarily reported by the original authors.

[d]ROR is the ratio of odds ratios (see text, Section 8.2.2). Corresponding dose is estimated for drinking water by assuming that the Cincinnati ratio of drinking water concentration to river water concentration (1:2) is constant for all cities in the study. A test for linear trend of ROR on dose is significant ($p<0.05$).

[e]Exposure: 8 hr/day, 5 days/wk.

TABLE 14-2

Reproductive Effects of Carbon Tetrachloride
from Subchronic Exposure

Route	Dose Level and Species	Exposure Duration	Biological Endpoint	Reference
Inhalation	622 mg/kg Rat	Daily; days 6-15 of gestation	Retarded fetal development	Schwetz et al., 1974
Inhalation	475 mg/kg Rat	Daily; for three generations	Reduced fertility	Smyth et al., 1936
Oral	2370 mg/kg Rat	2 or 3 days; during gestation	Reduced fertility	Wilson, 1954

TABLE 14-3

Summary Table for Mutagenicity Studies

Assay	Response	Reference
Ames test	Negative	McCann et al., 1975
E. coli reversion test	Negative	Uehleke et al., 1976
In vitro chromosome assay	Negative	Dean and Hudson-Walker, 1979
Yeast cells	Positive	Callen et al., 1980

TABLE 14-4

Carcinogenicity Studies Useful for Risk Assessment of Carbon Tetrachloride[a]

Species/ Strain	Number[b]	Dose[c] Regimen	Dose	Duration		Animals with Tumors		Tumor Type	Reference
				Exposure	Observation	Control	Treated		
Mice/ Imbred L	73 Treated 152 Control	2-3x/wk	0.04 mL (≈2100 mg/kg)	4 mo	7.0-7.5 mo	1%	47%	Liver tumor	Edwards et al., 1942
Mice/ $B6C3F_1$	200 Treated 40 Control	5x/wk in corn oil	1250 mg/kg 2500 mg/kg	78 wk	92 wk	8%	100% 97%	Hepato-cellular carcinoma	NCI, 1976

[a]Studies providing both a NOAEL and a LOAEL

[b]Includes males and females

[c]By gavage or gastric intubation

they not only provide all of the necessary quantitative information (e.g., number of animals in control and treated groups) but also include sufficient detail to demonstrate the high quality and defensibility of the research.

14.1.1. Ingestion. The only dose-related human data with complete quantitative information for oral ingestion (see Section 8.8.2.) are from an epidemiological study which found elevated serum creatinine levels following approximately 1-day exposure to carbon tetrachloride in drinking water. Several animal studies showed dose-related effects from carbon tetrachloride ingested via water, gavage or diet. Most of these studies involved a single dose (e.g., Korsrud et al., 1972). Acute effects (with increasing dose) included greater liver weight than normal, greater amount of liver fat, changes in enzyme levels or activity, some liver necrosis, reversible lung and kidney structural changes, lung lesions and behavioral abnormalities. One short-term (6-week) study in rats showed dose-related increases in total serum lipids and triglycerides and depressed weight gain (Alumot et al., 1976).

Several authors reported that carbon tetrachloride does not appear to be a teratogen but that it can affect reproduction following subchronic exposure. Oral administration of carbon tetrachloride to rats for either 2 or 3 days during days 7 to 14 of gestation was reported to cause a reduction in litter sizes and an increase in the number of fetal resorptions (Wilson, 1954).

Carbon tetrachloride has produced liver tumors in hamsters, mice and rats. A number of experiments have been conducted using mice of various strains ($B6C3F_1$, C3H, A and L) and different dosage regimens of carbon tetrachloride (Edwards et al., 1942; NCI, 1976). The types of tumors observed have included hepatomas, hepatocellular carcinomas and hyperplastic

hepatic nodules. Hyperplastic hepatic nodules were also induced in Syrian golden hamsters by oral doses of carbon tetrachloride (Della Porta et al., 1961). Effects induced by carbon tetrachloride in several strains of rats also include cholangiofibrosis, hepatic hyperplasia, hyperplastic hepatic nodules and hepatic carcinomas (Reuber and Glover, 1967, 1970).

14.1.2. Inhalation. The majority of the toxicity studies encountered in the available literature involved inhalation of carbon tetrachloride and included a wide range of exposure levels. One 70 minutes exposure study found decreased serum iron and altered serum transaminase levels, implicating liver damage (Stewart et al., 1961). A 2-year exposure in humans showed reversible nausea, anorexia, vomiting and epigastric discomfort (Kazantis and Bomford, 1960).

Effects from short-term exposure on animals appear to be dose-dependent and include: increased kidney and liver weights, reduction in activities of various enzymes, morphological and cellular changes in the liver and the lung, and Clara cell lesions in the lung. Dose-related effects from subchronic exposures are highly species-specific. For example, reversible liver damage was seen in the monkey at the same dose and duration which caused liver cirrhosis and increased mortality in the guinea pig. Other effects due to subchronic exposures include minor liver damage, weight loss and damage to the sciatic nerve.

Only one chronic inhalation animal study (of 10 months duration) was found in the literature. Liver damage was reported. The authors did not report liver tumors (Smyth et al., 1936).

When pregnant rats were exposed to carbon tetrachloride by inhalation on days 6 to 15 of gestation, the exposure resulted in decreased fetal body weights and lengths (Schwetz et al., 1974). In a three-generation inhala-

tion study involving rats, a dose of 238 mg/kg/day did not affect reproductive functions, while a dose of 475 mg/kg/day caused reduced litter sizes (Smyth et al., 1936). Single subcutaneous injection of pregnant animals with carbon tetrachloride has also been reported to produce histological and biochemical changes in the livers of the offspring; thus, Bhattacharyya (1965) concludes that carbon tetrachloride may be fetotoxic and can cross the placental barrier.

14.1.3. Dermal Exposure. Guinea pigs exposed to pure carbon tetrachloride in a skin painting experiment showed epidermal spongiosis and karyopyknosis, altered liver morphology and some liver necrosis (Kronevi et al., 1979).

14.1.4. Mutagenicity. Carbon tetrachloride has produced negative results in the Ames Salmonella test, in both the presence and the absence of microsomal activation (McCann et al., 1975) and in the Escherichia coli K12 test (Uehleke et al., 1976). Carbon tetrachloride produced negative results and was non-clastogenic in a chromosome assay using an epithelial-type cell line derived from rat liver which possessed intrinsic metabolizing activity (Dean and Hudson-Walker, 1979).

However, Callen et al. (1980) found carbon tetrachloride to be genetically active and cytotoxic in strain D yeast cells of Saccharomyces cerevisiae. In these cells, which contain a cytochrome P-450-dependent mono-oxygenase system, carbon tetrachloride caused increased frequencies of "gene conversion and mitotic recombination" and decreased cell survival.

14.2. SENSITIVE POPULATIONS

Studies on human sensitivity are limited. There is clinical evidence, discussed in Chapter 12, that two chemicals, isopropanol and ethanol, may potentiate carbon tetrachloride toxicity in humans. As described by Moon (1950), a repeated history of alcoholism in cases of fatal CCl_4 poisoning

may indicate a synergistic effect between alcohol and CCl_4. In other case reports, very obese and undernourished persons suffering from pulmonary diseases, gastric ulcers, liver or kidney diseases, diabetes or glandular disturbances seem especially sensitive to the toxic effects of CCl_4.

Supportive studies on rats suggest that older animals are more susceptible to the toxic effects of CCl_4 than are younger animals, and that males are more susceptible than females (Rueber and Glover, 1967). Chaturvedi (1969) examined age and sex as factors in CCl_4 toxicity. The findings revealed that female rats are less susceptible to the adverse effects of different hepatotoxic agents. Chaturvedi (1969) postulated that this was due to different hormonal and enzyme patterns and the lack of certain proteins in the female liver. The sex difference noticed in adult rats was not as apparent in young rats.

Nutritional status may also affect the degree of toxicity following exposure to carbon tetrachloride in rats. Gyorgy et al. (1946) exposed young rats on various diets to ≈300 mg/kg carbon tetrachloride in a gas chamber 7 hours/day, 5 days/week for 45 months. Animals were then sacrificed, and histopathological effects in the liver and kidneys were determined. When animals fed standard chow were compared to other groups, these effects were more severe in animals fed a diet high in lipid and low in carbohydrate, or a diet low in protein. Methionine appeared to protect against increased toxicity, particularly kidney damage, caused by low-protein diets (Gyorgy et al., 1946).

The interaction of carbon tetrachloride with other chemicals has resulted in an enhancement of the toxic effects produced in animals by either chemical alone. Exposure of animals to selected environmental carcinogens in combination with carbon tetrachloride has resulted in an increase in car-

cinogenicity. In addition, certain chemicals appear to increase the toxic effects of carbon tetrachloride on the liver and other organs of experimental animals.

14.3. QUALITATIVE HEALTH HAZARD ASSESSMENT

The assessment of human health risks, that is, the likelihood of certain adverse effects from given exposure scenarios, is hampered by the paucity of good dose-response data in humans. Of the three human studies discussed above, the acute epidemiological study involving oral ingestion (Sonich et al., 1981) and the acute inhalation study (Stewart et al., 1961) show serum alterations. The short-term inhalation study shows reversible, minor CNS and gastrointestinal effects (Kazantis and Bomford, 1960). In other instances, although several case reports document human effects, concentrations and exposures are not reported. Therefore, the prediction of toxic effects in humans and the determination of no-effect levels for subsequent use in a quantitative hazard or risk assessment, are primarily estimated from the many animal studies showing dose-related effects.

The major reported sublethal health hazards to humans from exposure to carbon tetrachloride are damage to the liver, lungs, kidneys and central nervous system. Less severe adverse effects include altered enzyme activities following ingestion and inhalation, gastrointestinal disturbances following inhalation, and epidermal damage following dermal contact. In animal studies, some effects are seen in areas distant from the contact interface. These include lung damage from oral ingestion, liver damage from inhalation and from dermal contact and, to a lesser degree, enzyme disturbances from inhalation and ingestion.

Teratogenic, mutagenic and carcinogenic effects have not been demonstrated in humans, and only carcinogenicity has been shown in experimental

animals. In addition, fetotoxicity and neonatal toxicity have been shown in rats (Schwetz et al., 1974; Bhattacharyya, 1965). Reproductive efficiency has also been affected in rats evidenced by reduced litter sizes (Smyth et al., 1936). Negative mutagenicity has been demonstrated in four studies including Salmonella typhimurium (McCann et al., 1975; Simmon and Tardiff, 1977), E. coli (Uehleke et al., 1976), and in a recently developed epithelial-type cell chromosome assay (Dean and Hudson-Walker, 1979). The single positive mutagenicity test in yeast cells, Saccharomyces cerevisiae, therefore requires further confirmation (Callen et al., 1980).

Carcinogenicity has been demonstrated in three experimental animal species, but predominantly several strains of rats and mice (NCI, 1976; Edwards et al., 1942; Reuber and Glover, 1970). Thus, carbon tetrachloride should be considered a potential human carcinogen, even though no definitive cause-and-effect human data exist.

Adverse effects on the biosphere from partial depletion of the ozone layer by carbon tetrachloride and the subsequent increase in UV flux are of concern for future research. The lack of stratospheric CCl_4 data and the uncertainties relative to the currently used, simplified transport models prevent reliable hazard assessment.

14.3.1. Animal Toxicity Studies Useful for Hazard Assessment. The preferred studies for hazard assessment are those which provide definite effect levels. Adverse effects are defined here as functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge. Adverse effects which are not carcinogenic are assumed to be threshold

phenomena. The threshold region of toxicity is estimated by evaluating four types of effect levels:

NOEL No-Observed-Effect Level: That exposure level at which there are no statistically significant increases in frequency or severity of effects between the exposed population and its appropriate control.

NOAEL No-Observed-Adverse-Effect Level: That exposure level at which there are no statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse.

LOAEL Lowest-Observed-Adverse-Effect Level: The lowest exposure level in a study or group of studies which produces statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

FEL Frank-Effect Level: That exposure level which produces unmistakable adverse effects, ranging from reversible histopathological damage to irreversible functional impairment or mortality, at a statistically significant increase in frequency or severity between an exposed population and its appropriate control.

Studies providing both a NOAEL and a LOAEL, therefore, are the most useful studies for hazard assessment.

The rat was the most commonly used animal in dose-response studies on carbon tetrachloride, with 12 satisfactory studies (as defined earlier) representing 22 dose-exposure groups (see Table 14-1). Korsrud et al. (1972) reported a single-dose LOAEL for rats of 20 mg/kg, virtually identical to the 6-week rat NOEL of 22 mg/kg given by Alumot et al. (1976). These results may be consistent. The infrequent, minor liver cell changes reported by Korsrud et al. may have been present in the Alumot et al. animals without affecting the serum levels monitored in the latter study. This illustrates the dependence of effect-level category on the endpoint investigated.

Extensive single-dose information on lung effects from oral ingestion was reported by Boyd et al. (1980), who studied two dose levels in both rats and mice, the lower in each case resulting in a "lung NOEL." Other studies which provide dose-related effect data for single oral exposures are listed in Table 14-1. The only subchronic oral study (6 weeks) provided a NOEL and two higher adverse effect levels for rats (Alumot et al., 1976).

Acute inhalation studies did not provide dose-related effect data. Intercomparison of four studies in rats showed a dose-dependent progression in severity of effects, with doses ranging from 300 to 285,000 mg/m^3 (Merkureva et al., 1979; Wong and DiStefano, 1966; Boyd et al., 1980; Chen et al., 1977). Useful subchronic inhalation data were provided by Prendergast et al. (1967), who used three dose levels (6.1 to 515 mg/m^3) including a NOEL, resulting in progressive effects on the liver of rats. The authors also applied identical dose levels to guinea pigs, the lowest also being a NOEL, with more severe effects resulting from the two higher levels. The only chronic inhalation study involved rats and guinea pigs and demonstrated effects from four dose levels administered for 10 months (Smyth et al., 1936). The effects in rats were predominantly on the liver, whereas in the guinea pigs the endpoint was mortality, dependent on dose and length of exposure.

The only dermal study providing dose-response information involved a single dose for short durations (0.3 and 16 hours) using guinea pigs (Kronevi et al., 1979). This study is most important for the duration-related effects on the liver, demonstrating definite effects of dermal exposures at a distant site.

14.3.2. Animal Carcinogenicity Studies. Liver tumors have been demonstrated in several species following oral exposure to carbon tetrachloride.

only a few studies, however, were designed to demonstrate the relation between tumor incidence and dose or exposure duration. Two studies provided definitive information on dose and incidence in controls and treated animals (see Table 14-4). Edwards et al. (1942) reported dose-related incidence of tumors for inbred strain L mice of two age groups, and NCI (1976) demonstrated significant tumor incidence for treated B6C3F$_1$ mice. It should be noted that in most of the animal studies showing carcinogenicity either the length of the study was too short or the dose level was too high for a dose-response estimation of lifetime exposure (NAS, 1978). The National Research Council recognizes this problem and for this reason uses the NCI (1976) bioassay for trichloroethylene (with CCl_4 as a positive control) for determining a carcinogenic risk estimate for carbon tetrachloride.

14.4. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT

14.4.1. Exposure. Carbon tetrachloride is persistent in air and ground water. Contamination of surface water and soil by carbon tetrachloride is not likely to present long-term hazards due to its rapid volatilization. However, large quantities of CCl_4 in bodies of cold water, such as lakes, are likely to remain submerged and be relatively stable, contaminating the body of water for several years. Carbon tetrachloride is readily absorbed through the lungs, but it is also rapidly exhaled; it is excreted through all routes, predominantly as the parent compound. The relative contributions of these factors to the bioavailability and body burden of CCl_4 in humans are not well defined. Also, experimental animals may differ substantially from humans in terms of oral and dermal efficiencies of absorption. Thus, application of human or animal pharmacokinetic information to quantitative human health hazard assessment does not seem feasible at this time. Consequently, the usable exposure information is from monitoring data of ambient CCl_4 levels in air, water and other liquids, and food.

14.4.2. Estimated Threshold No-effect Levels.* The dose-response data for carbon tetrachloride are quite limited, especially regarding effects on humans (Table 14-5). A human NOAEL for oral ingestion was reported as 0.2 mg/day (≈ 0.1 ppm) for 1-day exposure. The observed effect was a dose-related increase in the frequency of elevated creatinine levels in the study population. A human NOEL for inhalation was reported as 63 mg/m^3 (≈10 ppm) for 3-hour exposure. The monitored effects were serum enzyme and iron levels. Animal data are slightly more complete, with rat NOELs (not human equivalent) for inhalation ranging from 6.1 to 315 mg/m^3, depending on length of exposure. Insufficient information exists to allow estimation of NOAELs based upon long-term exposures.

In light of the uncertainties and inadequacies associated with the data base for CCl_4, particularly with the human NOEL and NOAEL given above, calculations of acceptable chronic exposure levels must be approached with caution. The lack of good absorption data is the main obstacle to accurate conversion of the animal exposure data to human equivalent exposures.

14.4.3. Carcinogenicity. Liver tumors have been shown to result from oral exposure to carbon tetrachloride by three species of animals. Although several authors note that toxic effects are concurrent with liver tumors, it has not been established that tissue damage is a necessary precursor to CCl_4 carcinogenesis. However, in view of the inconclusive nature of the presently available evidence for the mutagenicity of CCl_4, the upper bound of risk is presently regarded as having only limited plausibility.

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*Animal studies discussed herein indicate that carbon tetrachloride is a potential human carcinogen, and hence a no-effect level may not exist. These no-effect levels are supplied for comparison purposes.

TABLE 14-5

Reported No-Effect Levels for Toxicity of Carbon Tetrachloride[a]

Route	Exposure	Animal	No-Effect Level[b]	Lowest-Observable-Adverse-Effect Level[c]	Observed Effects	Reference
Oral	Single dose	Rat	d/	20 mg/kg	Liver cell changes	Korsrud et al., 1972
		Mouse	1600 mg/kg	4000 mg/kg	Widespread lung cell changes	Boyd et al., 1980
		Dog	116 mg/kg	797 mg/kg	Liver necrosis	Gardner et al., 1924
	Acute	Human[e]	0.2 mg/day	--	Elevated serum creatinine	Sunich et al., 1981
	Subchronic	Rat	22 mg/kg	40 mg/kg	Elevated serum lipids and triglycerides	Alumot et al., 1976
	Chronic	Rat	29 mg/kg	--	Reproductive performance and body weight	Alumot et al., 1976[f]

TABLE 14-5 (cont.)

Route	Exposure	Animal	No-Effect Level[b]	Lowest-Observable-Adverse-Effect Level[c]	Observed Effects	Reference
Inhalation	Acute	Human	63 mg/m^3	309 mg/m^3	Decreased serum iron and altered transaminase levels	Stewart et al., 1961
	Subchronic	Rat (6 wk)	6.1 mg/m^3	61 mg/m^3	Liver degeneration and fatty infiltration	Prendergast et al., 1967
		Rat (5 mo)	14 mg/m^3	--	Altered respiratory rates and sensitivity of respiratory enzymes to inhibitors	Rotenberg, 1978
		Guinea pig	6.1 mg/m^3	61 mg/m^3[e]	Increased mortality, liver damage	Prendergast et al., 1967
	Chronic	Rat	315 mg/m^3	630 mg/m^3	Liver cirrhosis	Smyth et al., 1936

[a]Extracted from Table 14-1.

[b]Includes no-observable-effect levels and no-observable-adverse-effect levels (see Table 14-1). These are the reported animal exposure levels and are not human equivalent exposure levels. Therefore, doses are not comparable and may not be directly applicable to the human exposure situation.

[c]Lowest or only adverse-effect level reported.

[d]No NOEL was reported for the rat in this study. Boyd et al. (1980) gives a rat NOEL of 3816 mg/kg; however, this is for lung effects only.

[e]The elevated serum creatinine levels are not considered to be an adverse effect. No human data exist showing dose-related, adverse effects from oral ingestion of carbon tetrachloride.

[f]One-year results inferred from a 2-year study; see discussion in Section 8.1.3.

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Appendix: Unit Risk Estimate for Cancer

Unit risk is defined as the lifetime cancer risk associated with continuous lifetime exposure to water or air containing a unit concentration of a pollutant in each respective media. Using the units of 1 µg/ℓ and 1 µg/m^3 for water and air, respectively, route-specific unit risk estimates are calculated for carbon tetrachloride (CCl_4). Upper bounds of risk have been estimated for individuals undergoing either of two hypothetical lifetime exposure situations: 1) continuous exposure to 1 µg CCl_4/m^3 of air, or 2) continuous exposure to 1 µg CCl_4/ℓ in drinking water. These estimates are based upon the results of the National Cancer Institute (NCI, 1976) bioassay for trichloroethylene, in which CCl_4 of unspecified purity, was used as the positive control. This bioassay examined the incidence of hepatocellular carcinoma in male mice. The effects of CCl_4 have been studied extensively in a number of other studies examining the effects of ingestion and inhalation exposures upon mice as well as rats, hamsters and dogs. However, upon evaluation of these studies, either the length of the study was too short or the dose level was too high to obtain a dose-response estimation of lifetime exposure (NAS, 1978).

In this NCI study, male mice received CCl_4 by gavage at 1250 mg/kg and 2500 mg/kg 5 days/week for 78 weeks (546 days). Liver tumors were observed at 92 weeks (644 days) when the experiment was terminated. Using the reported data, the parameters of the extrapolation model are:

Dose (mg/kg/day)	Liver Cancer Incidence (no. affected/no. examined)
0 (pooled venicle control)	5/77 (6%)
1250	49/49 (100%)
2500	47/48 (98%)

le = 78 weeks = 546 days
Le = 92 weeks = 644 days
L = 92 weeks = 644 days
w = 0.028 kg

As given in the Guidelines for determining the impact of pollutants upon human health (45 FR 79351), the following procedure is used to calculate the carcinogenic potency (q_1^*) of CCl_4 for humans. Lifetime time-weighted average daily doses (d) for animals are computed:

$$d = \text{dose (mg/kg/day)} \times 5/7 \times 1e/Le$$

Thus,

$$d_{low} = 1250 \text{ mg/kg/day} \times 5/7 \times 546/644 = 754 \text{ mg/kg/day}$$

$$d_{high} = 2500 \text{ mg/kg/day} \times 5/7 \times 546/644 = 1508 \text{ mg/kg/day}$$

Using these values, the carcinogenic potency (q_1^*) for animals is computed via the linearized multistage model. The linearized form of the multistage model can be expressed:

$$P = 1 - \exp[-q_1^* \times d + \text{higher order terms in dose}]$$

This is derived and discussed in the Guidelines as cited above. For low doses, the higher order terms are negligible compared to the q_1^*d term. The animal q_1^* is then converted to a q_1^* for humans using the body weights of 0.028 kg for mice and 70 kg for humans with the assumption that doses producing equivalent effects are calculated in terms of mg chemical/surface area of animal.

Thus, the CCl_4 carcinogenic potency factor for humans is estimated:

$$q_1^* = 8.275 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$$

From this factor the carcinogenic potency of CCl_4 can be expressed in terms of exposure via water or air as follows.

Unit Risk Estimate for Humans from Exposure to Carbon Tetrachloride in Water.

To estimate the risk corresponding to the concentration of 1 µg CCl_4/ℓ water, the effective dose in terms of mg/kg/day corresponding to

1 μg/ℓ must first be estimated. Assuming a water intake of 2 ℓ/day and a 100% absorption rate by ingestion for a 70 kg human:

$$d_{water} = [2\ \ell/day \times 1.00 \times 1/(70kg)] \times 10^{-3}\ mg/\ell$$
$$= 2.86 \times 10^{-5}\ mg/kg/day$$

Using the multistage model, the upper bound of the risk corresponding to d_{water} is estimated:

$$P(d_{water}) = 1 - \exp[-8.275 \times 10^{-2} \times 2.86 \times 10^{-5}]$$
$$= 0.236 \times 10^{-5}$$

Therefore, the unit cancer risk estimate for humans from exposure to CCl_4 in water, that is, the risk corresponding to 1 μg/ℓ, is 0.236×10^{-5}.

Unit Risk Estimate for Humans from Exposure to Carbon Tetrachloride in Air.

To estimate the risk corresponding to the concentration of 1 μg CCl_4/m^3 air, the effective dose in terms of mg/kg/day corresponding to 1 $\mu g/m^3$ must first be estimated. Assuming an air intake of 20 m^3/day and a 40% absorption rate by inhalation for humans (as recommended in this document), this effective dose can be estimated for a 70 kg human:

$$d_{air} = [20\ m^3/day \times 0.40 \times 1/(70kg)] \times 10^{-3}\ mg/m^3$$
$$= 1.14 \times 10^{-4}\ mg/kg/day$$

Using the multistage model, the upper bound of the risk corresponding to d_{air} is estimated:

$$P(d_{air}) = 1 - \exp[-8.275 \times 10^{-2} \times 1.14 \times 10^{-4}]$$
$$= 0.945 \times 10^{-5}$$

Therefore, the unit cancer risk estimate for humans from exposure to CCl_4 in air is directly obtained from the potency such that the risk corresponding to 1 $\mu g/m^3$ is 0.945×10^{-5}.

In this calculation, the linearized multistage model is used to give the following estimates of a plausible upper bound of lifetime cancer risk:

0.236×10^{-5} for a person continuously exposed to 1 µg CCl_4 per liter of water, or

0.945×10^{-5} for a person continuously exposed to 1 µg CCl_4 per cubic meter of air.

Because of the uncertainties in both the qualitative and quantitative aspects of risk assessment, the actual cancer risks may be lower than those indicated above, which should be regarded as plausible upper-limits and may approach zero. However, in view of the inconclusive nature of the presently available evidence for the mutagenicity of CCl_4, the upper bound of risk is presently regarded as having limited plausibility.

The above information provides route-specific cancer risk estimates associated with exposure to given units of CCl_4. These estimates may be conservative due to the mathematical model employed. Nevertheless, unit risks for cancer are defined for independent water and air exposures in that their computation assumes 100% of the insult is via the stated route.

When information is available to indicate the occurrence of concurrent exposures, two steps should be considered in estimating one risk. First, the risks associated with independent exposures can be adjusted for concentration, if known to be different from that defined by the unit risk estimate. This adjustment can be done by simple multiplication or division and is justified in that exposure is in the low dose region where risk is directly proportional to dose. For example, if it is known that the concentration in air is 1.5 µg/m^3 then the upper bound of the risk associated with 1 µg/m^3, 0.95×10^{-5}, can be multiplied by 1.5 to obtain:

$$(0.945 \times 10^{-5})\ (1.5) = 1.42 \times 10^{-5}$$

Thus, it can be inferred that the upper bound of the risk associated with the concentration of 1.5 $\mu g/m^3$ CCl_4 in air is 1.42×10^{-5}.

Once such an adjustment is made, if necessary, the additivity assumption can be used to calculate the risk associated with concurrent exposures via two routes. It is a general recommendation to use the additivity assumption which is made since the available data on CCl_4 are limited and do not allow the presentation of a defensible alternative. As new information becomes available, other alternatives should be considered. Here, the additivity assumption is that the risk associated with exposure to a given chemical via two routes concurrently is roughly the sum of the risks associated with each independent route-specific exposure. Since interactions between the concurrent routes of intake cannot be quantified, uncertainty surrounds the resulting risk estimate that is derived from the concurrent risks.

In applying the assumption of additivity, the risks rather than the doses associated with each route are added, but the mere summation of these risks is presently justifiable only at low doses. Furthermore, the amounts of 1 $\mu g/\ell$ and 1 $\mu g/m^3$ are concentrations in water and air, respectively, not doses. To state an example, the risk of 0.236×10^{-5} is associated with a lifetime exposure to 1 μg CCl_4/ℓ water. The dose can be estimated by assuming consumption of 2 ℓ water/day over the lifetime. Thus, the daily dose corresponding to a concentration 1 $\mu g/\ell$ water would be 2 ℓ/day x 1 $\mu g/\ell$ = 2 μg/day.

In conclusion, based upon the data for CCl_4 given in the NCI bioassay for trichloroethylene, in which CCl_4 was used as a positive control, the upper bounds of route-specific unit risk estimates for cancer are computed for exposures via water and air:

> The unit cancer risk estimate for humans from exposure to a concentration of 1 µg CCl_4/ℓ water is 0.236×10^{-5}.
>
> The unit cancer risk estimate for humans from exposure to a concentration of 1 µg CCl_4/m^3 air is 0.945×10^{-5}.

However, each upper bound of risk is presently regarded as having limited plausibility due to the inconclusive nature of the available evidence for the mutagenicity of CCl_4. Furthermore, because of the uncertainties in both the qualitative and quantitative aspects of risk assessment, the actual cancer risks may be lower than those indicated above and may approach zero.